REMARKS

The August 4, 2009 Official Action has been carefully considered. In view of the amendment presented herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset, it is noted that a shortened statutory response period of three (3) months was set in the August 4, 2009 Official Action. The initial due date for response, therefore, was November 4, 2009. A petition for a one (1) month extension of the response period is presented with this amendment and request for reconsideration, which is being filed within the one (1) month extension period.

It is also noted that preliminarily that the restriction requirement set forth in the preceding Official Action has been repeated and made final. Applicants once again respectfully submit that this requirement is improper for the reasons presented in response to the preceding Official Action. Furthermore, applicants reiterate that the election of the subject matter of claims 44 and 46-48 for examination in this application is without prejudice to their right to file one or more continuing applications, as provided under 35 USC §121, on the subject matter of the non-elected claims.

In the August 4, 2009 Official Action, claims 44 and 46-48 have been objected to as being dependent on withdrawn claim 1.

Turning to the substantive aspects of the August 4 Official Action, claims 44 and 46-48 stand rejected under 35 USC §112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter regarded as the invention. Specifically, the polyethylenimine formula is deemed indefinite based on the lack of a positive charge shown in association with the quaternary nitrogens, an alleged lack of clarity as to what the proportions of each subunit in a species of the polymer genus should be and the characterization of hydrogen as "hydrophobic" in the definition of the "Z" substituent. Claim 44 is considered indefinite because, according to the examiner, it is unclear whether (i) the claim requires the further addition of a pharmaceutically acceptable carrier and (ii) the recitation following the words "may represent" is required. Claims 47 and 48 are considered indefinite due to the wording "such as" which appears in each claim.

Claims 44 and 46-48 have been further rejected under 35 USC §103(a) as allegedly unpatentable over D. Wang, Biomacromolecules, 3: 1197-1207 (2002) (hereinafter "the Wang publication"), considered in view of U.S. Patent 4,528,184 to Kurono et al. (hereinafter "Kurono '184") and U.S. Patent Application Publication No. 2001/0014354 of Yokoyama et al. (hereinafter "Yokoyama '354").

The Wang publication is cited for its disclosure of branched poly(ethyleneimine)-cholesterol (PEI-Chol) water-soluble, lipopolymers which are described as suitable for gene delivery, having the possibility of efficient endosomal release.

Kurono '184 is relied on for its disclosure of polymer-metal complexes containing quaternary nitrogen atoms and their use as a pharmaceutical product.

Yokoyama '354 relates to a drug-polymer micelle composition and process for its production. According to the disclosure of Yokoyama '354, the process described therein produces drug-polymeric micelle compositions comprising water-scarcely soluble drugs, including carcinostatic agents such as adrimacyn, paclitaxel, docetaxel and the like.

Based on the disclosures of the above-mentioned references, the examiner contends, at page 9 of the August 4 Official Action, that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to combine Yokoyama '354 and Kurono '184 with the Wang publication and produce the instantly claimed invention because the poorly soluble drugs taught by Yokoyama '354 would allegedly have been suitable for the gene delivery micellar composition of the Wang publication and the increased charge of the nitrogen atoms in the PEI-type polymers of the Wang publication would have increased the interaction with the poorly water soluble drug, providing an increased loading of the drug into the micelle. The examiner further asserts in this regard that the charges of the quaternary nitrogen atoms would have allowed the formation of potentially more stable and/or efficacious salts of poorly soluble drugs.

The foregoing objection and rejections constitute all of the grounds set forth in the August 4, 2009 Official Action for refusing the present application.

In accordance with the present amendment, new claims 49-56 are presented for consideration. New claims 49, 52 and 54 correspond substantially to original claims 46 (as well as 47), 44 and 48, respectively. Claim 49 also finds support in Figure 1. Claim 53 finds support at page 9, lines 37 and 38 of the present specification. New claims 50-52 are supported at page

3, lines 33-37 of the present specification, whereas new claims 55 and 56 claim subject matter set forth originally in claim 48.

No new matter has been introduced into this application by reason of the present amendment, entry of which is respectfully requested.

As a result of the foregoing amendment, the above-noted objection and the 35 USC §112, second paragraph, rejection of claims 44 and 46-48 are believed to be overcome. As to the latter, any indefiniteness or lack of clarity that may have been engendered by the original wording of claims 44 and 46-48 has now been eliminated. In this connection, the assertion that the α, β and γ subunits of the claimed polyethylenimine lack clarity is unfounded. The scope of each subunit is plainly stated in claim 49, along with the recitation that "α+β+γ=100%". Although the claim recitation in question may be broad, it is certainly not indefinite. It is also noteworthy with respect to this rejection that the claim language in question is recited in the allowed claims of European Patent 1,543,063 B1 (copy attached), which was granted on applicants' corresponding European patent application.

Thus, the only matter remaining to be addressed is the rejection of claims 44 and 46-48 for alleged obviousness based on the combined disclosures of the Wang publication, Kurono '184 and Yokoyama '354, as set forth in the August 4, 2009 Official Action. For the reasons given below, the last-mentioned ground of rejection is respectfully traversed.

A. The Impropriety of the 35 USC §103(a) Rejection of Claims 44 and 46-48 as Allegedly Unpatentable Over the Combined Disclosures of the Wang Publication, Kurono '184 and Yokoyama '354

It is well-settled that the examiner has the initial burden of establishing a *prima facie* case of obviousness under 35 USC §103. *Ex parte Wolters,* 214 USPQ 735 (Bd. Apps. 1979). Furthermore, when a new composition is alleged to be obvious based on a combination of prior art references, there must be an apparent reason to modify compositions of the prior art in the fashion set forth in the claims in question. *Ex parte Whalen,* 89 USPQ2d 2008 (BPAI 2008), citing *KSR International Co. v. Teleflex, Inc.,* 82 USPQ2d 1385 (2007).

In the present case, the 35 USC §103(a) rejection of claims 44 and 46-48 based on the combined disclosures of the Wang publication, Kurono '184 and Yokoyama '354 is improper

because it clearly lacks the rational basis required to support the legal conclusion that the pharmaceutical drug delivery composition of the rejected claims would have been *prima facie* obvious.

Applicants' invention relates to novel polyethylenimine (PEI) polymers having hydrophobic and hydrophilic substituents that make the polymer effective for delivering poorly soluble drugs. The invention cannot reasonably be considered *prima facie* obvious over the combination of the Wang publication, Kurono '184 and Yokoyama '354, as none of these references disclose or suggest a polyethyleneimine polymer which has the arrangement of subunits called for in the formula of claim 44 (now incorporated in claim 49) and which possesses solubilizing and absorption enhancing properties, thereby facilitating delivery of poorly soluble drugs.

It is conceded, at page 8 of the August 4, 2009 Official Action, that the disclosure of the Wang publication is deficient as it lacks any teaching of the presence of quaternary nitrogens in the PEI-Chol lipopolymers described therein, or the use of such lipopolymers for the delivery of poorly water soluble drugs. These are not simply "deficiencies", as intimated by the examiner, but constitute a teaching away from the present invention. Indeed, the PEI-Chol lipopolymers described in the Wang publication have nothing at all to do with the delivery of drugs that are poorly soluble in aqueous solutions, as called for in original claim 47 and in the presently amended claims. On the contrary, plasmid DNA, which the PEI-Chol lipopolymers of the Wang publication are designed to deliver, is a highly charged molecule and thus completely soluble in aqueous solutions. Thus, viewed objectively, the artisan of ordinary skill would not consider the disclosure of the Wang publication, relating to the delivery of highly charged, water-soluble plasmid DNA by means of PEI-Chol lipopolymers, as being of any assistance when the objective sought is a delivery system for drugs that are poorly soluble in aqueous solvents.

In view of the admitted deficiencies in the disclosure of the Wang publication, it is incumbent upon the examiner, in order to meet the PTO's burden of proof under §103, to cite additional prior art that not only discloses the elements that are conceded to be missing from the Wang publication, but also provides a plausible reason that would motivate one of ordinary skill in the art to modify the composition of Wang et al. to include such missing elements and thus arrive at the claimed invention. The examiner's reliance on Yokoyama '354 and Kurono '184 as allegedly providing such teachings is plainly misplaced.

The examiner's assessment of the general teaching of Yokoyama '354 appears to be accurate as far as it goes. The examiner fails to mention, however, that there is no teaching or suggestion in Yokoyama '354 regarding the use of polyethylenimine polymers of any kind in producing the polymeric micelle compositions disclosed therein.

It is settled law that the disclosure of a broad chemical genus, such as that in Yokoyama '354, does not render obvious any species that happens to fall within it. *In re Jones,* 21 USPQ 1941, 1943 (Fed. Cir. 1992). Moreover, silence in a reference is not a proper substitute for the adequate disclosure of facts from which a conclusion of obviousness may justifiably follow. *In re Burt,* 148 USPQ 548 (CCPA 1996). Simply stated, the disclosures of the Wang publication and Yokoyama '354, considered together, provide no reason to incorporate the "water-scarcely soluble drug" of the latter in the PEI-Chol lipopolymers of the former, notwithstanding the examiner's assertion to the contrary.

Furthermore, Yokoyama '354 specifies that drug loaded polymeric micelles are only formed via the dissolution of the water insoluble drug and the polymer in an organic solvent, followed by the formation of an oil in water emulsion and the removal of the organic solvent by evaporation. See page 5, column 2, lines 18-33 and Examples 1-15. The polymers of the present invention are distinguishable from those in Yokoyama '354 in that no organic solvent is required to prepare drug loaded particles. See the present specification at page 15, lines 10-15. Residual chloroform as mentioned by Yokoyama '354 (page 4, column 2, lines 47-48) may be detrimental to the pharmaceutical composition. The exclusion of such organic solvents in the formulation of applicants' amphiphilic poly(ethylenimime)-drug loaded particles is a further advantageous feature of the invention. Advantages that are inherent in an invention need not be recited in the claims, but must nonetheless be considered in determining patentability. *In re* Estes, 164 USPQ 519 (CCPA 1970).

Even if the combination of features referred to by the examiner would have suggested itself to the artisan of ordinary skill based on the disclosures of the Wang publication and Yokoyama '354 (which applicants vigorously dispute), the resulting composition would still not satisfy all of the recitations of the rejected claims. Specifically, neither the Wang publication nor Yokoyama '354 teach or suggest a drug-containing composition comprising a polyethylenimine polymer including units in which a nitrogen is quaternized, as in applicants' elected species QCPAI2, for example.

The disclosure of Kurono '184 does not compensate for this additional fundamental deficiency in the disclosures of both the Wang publication and Yokoyama '354.

Kurono '184 relates to improvements in substances that lower levels of cholesterol in the serum and liver by disturbing the adsorption of bile acids, i.e., metabolites of cholesterol in the digestive tract, such substances including cross-linked polymers formed by introducing quaternary nitrogen atoms into a cross-linked polymer of styrene and divinyl benzene, as well as tetraethylenepentamine and epichlorohydrin. These polymer substances are described in Kurono '184 to be effective as hypercholesterolemia treating agents. However, it is pointed out that the use of these agents is accompanied by side effects, such as constipation, which "still remain as problems to be solved". See column 1, lines 6-30 of Kurono '184. The shortcomings of the prior art polymeric hypercholesterolemia treating agents are further explained on the basis that, as chelators, such polymers frequently "adsorb microelements which are essential for human bodies, when orally administered". See column 3, lines 1-2 of Kurono '184.

The solution to the above-mentioned problem, according to Kurono '184, is to form a cross-linked polymer that "can be converted to a polymer-metal complex quantitatively by quaternarization". Column 3, lines 39-42. Such polymer-metal complexes are said to have more stable structure due to the metal chelate bond formation, as compared to the corresponding non-metal chelate polymer of the prior art. As a result of preparing the polymer-metal complexes in this way, they "do not adsorb essential metal ions, when administered orally. See Example 7 of Kurono '184.

Considering the entire disclosure of Kurono '184, it is clear that one reason for quaternarizing the cross-linked polymer was to allow for quantitative conversion to the desired polymer-metal complex, so as to avoid adsorption of essential metal ions in recipients upon oral administration of these agents. No such problem concerning undesirable adsorption of essential metal ions is mentioned in association with the polymer compositions described in either the Wang publication or Yokoyama '354. Consequently, there would be no reason for making the polymer compositions of the Wang publication or Yokoyama '354 in the manner described in Kurono '184.

Another reason for incorporating quaternary ammonium groups into the polymer-metal complex of Kurono '184 is to improve their absorption of cholic acid (an acidic compound that would bind to the quaternary ammonium groups, as noted at column 3, lines 51-53; column 8,

Table 1). The examiner is plainly mistaken in stating, at page 9, lines 3-9 of the August 4, 2009 Official Action that quaternary ammonium groups increase the interaction between a poorly water soluble drug and the polymer. On the contrary, quaternary ammonium groups are hydrophilic and a poorly water soluble drug, being hydrophobic , would not associate with the quaternary ammonium groups, which would hydrogen bond with water, thus preventing such association. Consequently, these two chemical species would never bind.

The examiner's observation at page 9, lines 9-11 of the August 4, 2009 that charged quaternary ammonium groups would bind to salts of poorly soluble drugs has absolutely no relevance to the current invention. Salt forms of poorly soluble drugs are water soluble typically via their ability to hydrogen bond with water. In fact, salt formation is a well known method for increasing the water solubility of drugs (Florence AT and Attwood D, 2006, Physicochemical Principles of Pharmacy, Pharmaceutical Press, London, pp 161-162). Drugs with good water solubility are not encompassed by the claims as amended.

It is also noteworthy that the quaternarized polymer-metal complexes disclosed in Kurono '184 do <u>not</u> function as a carrier for a drug or therapeutic agent as the complexes themselves are therapeutic agents. As such, there would be no reason to incorporate any feature of the polymer-metal complex of Kurono '184 into the polymer compositions of the Wang publication or Yokoyama '354, which are intended <u>as carriers of additional therapeutic agents.</u>

The resolution of the obviousness/non-obviousness issue in this case must be in accordance with the relevant standards established by the PTO Board of Patent Appeals and Interferences (BPAI) in the aftermath of *KSR, supra.* The examiner has been conspicuously remiss in this regard, for it is impossible to reconcile the finding of obviousness herein with the BPAI's disposition of the appeal in *Whalen, supra.* As stated in *Whalen*:

> The *KSR* Court noted that obviousness cannot be proven merely by showing that the elements of a claimed device were known in the prior art; it must be shown that those of ordinary skill in the art would have had some 'apparent reason to combine the known elements in the fashion claimed' [citation omitted]. *Id.* at 1084.

The BPAI's treatment of the non-obviousness issue in *Whalen* is entirely consistent with prior decisions, such as *Ex parte Levengood,* 28 USPQ2d 1300 (BPAI 1993). In reversing a §103 rejection based on a combination of prior art references, the BPAI in *Levengood* stated:

> [T]he only suggestion for the examiner's combination of the isolated teachings of the applied references improperly stems from appellant's disclosure and not from the applied prior art. At best, the examiner's comments regarding obviousness amount to an assertion that one of ordinary skill in the relevant art would have been able to arrive at appellant's invention because he had the necessary skills to [do so]. This is an inappropriate standard for obviousness. That which is within the capabilities of one skilled in the art is not synonymous with obviousness. That one can reconstruct and/or explain the theoretical mechanism of an invention by means of logic and sound scientific reasoning does not afford the basis for an obviousness conclusion unless that logic and reasoning also supplies sufficient impetus to have led one of ordinary skill in the art to combine the teachings of the references to make the claimed invention.
>
> . . . [A]n examiner cannot establish obviousness by locating references which describe various aspects of a patent applicant's invention without also providing evidence of the motivating force which would impel one skilled in the art to do what the patent applicant has done [Emphasis added; citations omitted].

As in *Levengood*, the references cited as evidence of obviousness in this case fall far short of providing the apparent reason required to combine their elements in the manner claimed by applicants herein.

Furthermore, because the Wang publication teaches away from applicants' pharmaceutical composition as claimed in new claims 49-56, for the reasons set forth above, the combination of the Wang publication, Yokoyama '354 and Kurono '184 cannot render those claimed *prima facie* obvious. See *In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993) (reversing the PTO's obviousness determination where one of the cited references taught away from the claimed invention).

The preceding remarks have primarily addressed patentable differences between the pharmaceutical composition of claims 46 and 47 (now set forth in independent claim 49)and the combined disclosures of the Wang publication, Yokotyama '354 and Kurono '184. Claims 50-56 directly or indirectly from independent claim 49. It is axiomatic that any claim dependent from a non-obvious claim is also non-obvious. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988).

Lastly, the examiner is correct in presuming in the paragraph bridging pages 5-6 of the August 4, 2009 Official Action that the subject matter of the various claims was commonly owned at the time the inventions covered thereby were made.

Given that the prior art references cited in support of the present §103 rejection of fail to teach or suggest applicants' pharmaceutical composition claimed in claims 44 and 46-48, no evidence of a surprising or unexpected result need be presented. *In re Lunsford,* 148 USPQ 721 (CCPA 1966).

In summary, the §103 rejection of claims 44 and 46-48 based on the combined disclosures of the Wang publication, Yokoyama '354 and Kurono '184 is improper and should be withdrawn upon reconsideration.

B. **Conclusion**

In view of the present amendment and the foregoing remarks, it is respectfully requested that the objection and rejections set forth in the August 4, 2009 Official Action be withdrawn and that this application be passed to issue, and such action is earnestly solicited.

Respectfully submitted,

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Attachment:
1. EP 1,543,063 B1

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(54) DRUG DELIVERY

ARZNEIMITTELVERABREICHUNG

LIBERATION DE MEDICAMENTS

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WO-A-02/30468 **WO-A-99/43752**
US-A- 5 338 532 **US-A- 5 681 543**

- **NÖDING, G.; HEITZ, W.: "Amphiphilic polyethyleneimines based on long-chain alkyl bromide" MACROMOLECULAR CHEMISTRY AND PHYSICS, vol. 199, 1998, pages 1637-1644, XP002268918 cited in the application**

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Description

Field of Invention

[0001] This invention relates to the delivery of drugs. In particular, this invention relates to the oral delivery of poorly soluble drugs using novel amphiphilic polymers with both solubilising and absorption enhancing properties.

Background of Invention

[0002] The oral delivery of poorly soluble drugs is usually accomplished with oil based formulations such as microemulsions (Dunn, C.J., Wagstaff, A.J., Perry, C.M., Plosker, G.L., Goa, K.L.,2001, Cyclosporin - An Updated Review of the Pharmacokinetic Properties, Clinical Efficancy and Tolerability of a Microemulsion-Based Formulation Neoral R(1) in Organ Transplantation, Drugs 61: 1957 - 2016; and Porter, C.J.H., Charman, W.N., 2001, In vitro Assessment of Oral Lipid Based Formulations, Advanced Drug Delivery Reviews 50: S127-S147) or low molecular weight surface active agents (BalandraudPieri, N., Queneau P.E., Caroli Bosc, F.X., BertaultPeres, P., Montet, A.M., Durand, A., Montet, J.C. 1997, Effects of Tauroursodeoxycholate Solutions on Cyclosporin and Bioavailability in Rats, Drug Metabolism and Disposition 25: 912-916; Guo, J.X., Ping, Q.N., Chen, Y. 2001, Pharmacokinetic Behaviour of Cyclosporin A in Rabbits by Oral Administration of Lecithin Vesicle and Sandimmun Neoral, International Journal of Pharmaceutics 216: 17-21). Poorly soluble drugs are those drugs that are identified in the British Pharmacopoeia as "practically insoluble" (Medicines Commission, British Pharmacopoeia, The Stationary Office, London, 1998). Such drugs have an aqueous solubility of less than 0.1mg per millilitre of solvent (such as water) at a temperature of about 15°C - 20°C.

[0003] Previous attempts to promote oral absorption of poorly soluble drugs such as cyclosporin, have involved the use of oil and/or surfactant (Dunn, C.J., Wagstaff, A.J., Perry, C.M., Plosker, G.L., Goa, K.L.,2001, Cyclosporin - An Updated Review of the Pharmacokinetic Properties Clinical Efficacy and Tolerability of a Microemulsion-Based Formulation Neoral R(1) in Organ Transplantation, Drugs 61: 957 - 2016; and Porter, C.J.H., Charman, S.A., Williams, R.D., Bakalova, M.B., Charman, W.N., 1996, Evaluation of Emulsifiable Glasses for the Oral Administration of the Cyclosporin in Beagle Dogs, International Journal of Pharmaceutics 141: 227-237), bile salt (BaladraudPieri, N., Queneau, P.E., CaroliBosc F.X., BertaultPeres, P., Montet, A.M., Durand, A., Montet, J.C., 1997, Effects of Tauroursodeoxycholate Solutions on Cyclosporin and Bioavailablity in Rats, Drug Metabolism and Disposition 25:912-916), phospholipid based systems (Guo, J.X., Ping, Q.N., Chen, Y., 2001, Pharmacokinetic Behaviour of Cyclosporin A In Rabbits by Oral Administration of Lecithin Vesicle and Sandimmun Neoral, International Journal of Pharmaceutics 21: 17 - 21; and Leigh, M., Hoogevest, P.V., Tiemiessem, H., 2001 Optimising the Oral Bioavailablity of the Poorly Water Soluble Drug Cyclosporin A Using Membrane Lipid Technology, Drug Delivery and Sciences 1: 73-77) or cyclodextrins (Miyake, K., Arima, H., Irie, T., Hirayma, F., Uekama, K., 1999, Enhanced Absorption of Cyclosporin A by Complexation with Dimethyl-Beta-Cyclodextrin in Bile duct-Cannulated and Non-Cannulated Rats, Biological and Pharmaceutical Bulletin 22: 66-72). Although a nanocapsule formed during in-situ polymerisation has also been proposed for cyclosporin delivery, this technique has difficulties in delivering the drug (Bonduelle, S., Carrier, M., Pimienta, C., Benoit, J.P., Lenaerts, B., 1996, Tissue Concentration of Nanoencapsulted Radiolabelled Cyclosporin Following Peroral Delivery in Mice or Opthalmic Application in Rabbits, European Journal of Pharmaceutics and Biopharmaceutics, 42: 31 - 319).

[0004] Cyclosporin is a lipophilic immunosuppressant used to treat transplant and autoimmune disease patients. Cyclosporin is poorly soluble in a variety of solvents and is currently administered as micro-emulsion formulation.

[0005] Nöding & Heitz (Macromolecular Chemistry and Physics, 199: 1637-1644, 1998) describes the synthesis of polyethylenimine polymers with tertiary nitrogen atoms within the chain in a reaction in which branched polyethylenimines are created by the reaction of polyethylenimine with an alkyl bromide.

[0006] US 5681543 discloses polymer complexes for use as diagnostic complexing agents, including polymers derived from tertiary polyethylenimines. The polymers are used to complex metal ions for use in MRI.

[0007] US 5338532 discloses dendrimer molecules including dendrimers based on tertiary amines.

[0008] WO 02/30468 describes cationic polymers including polyethylenimines that are used to complex nucleic acid for delivering the nucleic acid into cells.

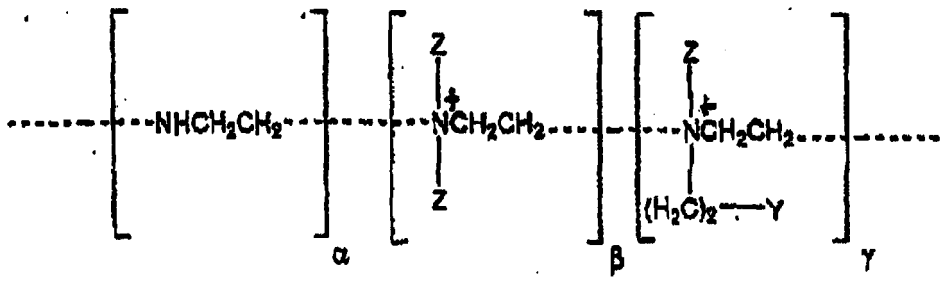
[0009] CA 2321200 describes a number of polyethylenimine products having two or more hydrophobic groups for complexing nucleic acid.

[0010] It is an object of embodiments of the present invention to obviate or mitigate at least one or more of the aforementioned problems.

[0011] It is a further object of embodiments of the present invention to improve delivery of poorly soluble drugs to a recipient.

Summary of the Invention

[0012] Accordingly, in a first aspect, the present invention provides a pharmaceutical composition of a poorly soluble drug and amphiphilic polyethylenimine polymer, wherein the drug has an aqueous solubility of less than 0.1 mg per millilitre of solvent at a temperature of about 15-20°C, wherein the polymer contains hydrophilic substituents that comprise quaternary nitrogen groups and hydrophobic substituents as represented by the formula:



wherein

α is between 0 to 90%;
β is between 0 to 100%;
γ is between 0 to 50%;
and α + β + γ = 100%; and

and further wherein:

each Z group is independently hydrogen or a hydrophobic substituent, wherein the hydrophobic substituent is a linear or branched, substituted or unsubstituted or a cyclic group; and
Y is a hydrophilic substituent.

[0013] In a further aspect, the present invention provides the sse of a polyethylenimine polymer as defined above for the preparation of a medicament wherein the polymer is formulated with a poorly soluble drug, wherein the drug has an aqueous solubility of less than 0.1 mg per millilitre of solvent at a temperature of about 15-20°C.

[0014] It should be understood that the monomer units identified with α, β and γ may form any arrangement in the polyethylenimine polymer. The arrangement of the α, β and γ units may therefore be random or in a block copolymer form such as αβγαβγαβγ etc. This is identified above by the dashed line between the different monomer units.

[0015] The polyethylenimine polymer may be linear or branched.

[0016] The ratios for α, β, γ are numerical ratios.

[0017] Typically, the Z groups may independently be selected from any of the following hydrophobic substituents: an alkyl, an alkenyl, and alkynyl, an aryl, an acyl, a hydroxy alkyl, a hydroxy acyl, polyethylene glycol or any sugar.

[0018] The Z groups may independently be any linear or branched, substituted or unsubstituted, or cyclo form of the following alkyl, alkenyl, alkynyl, aryl, acyl, hydroxy alkyl, hydroxy acyl, polyethylene glycol or any sugar groups: C_1 - C_{20}; C_1 - C_{12}; C_1 - C_6 or C_1.

[0019] The Z groups may be C_1 - C_4 linear alkyl groups.

[0020] Y may represent any of the following: $-NH_2$; -NHA; $-N^+R_1R_2R_3$; and $-N^+R_1R_2A$.

[0021] R_1, R_2, or R_3 may be selected from any of the following substituents: an alkyl, an alkenyl, an alkynyl, an aryl, an acyl, a hydroxy alkyl, a hydroxy acyl, polyethylene glycol or any sugar.

[0022] R_1, R_2 and R_3 may independently be any linear or branched, substituted or unsubstituted, or cyclo form of the following alkyl, alkenyl, alkynyl, aryl, acyl, hydroxy alkyl, hydroxy acyl, polyethylene glycol or any sugar groups: C_1 - C_{20}; C_1 - C_{12}; C_1 - C_6 or C_1.

[0023] Typically, R_1, R_2 and R_3 are C_1 - C_4 linear alkyl groups.

[0024] All of R_1, R_2 and R_3 may be CH_3.

[0025] Conveniently there may be between 1 and a maximum of 3 R substituents on any single nitrogen. This allows for primary, secondary and tertiary amines.

[0026] The groups A may be selected from any of the following linear or branched, substituted or unsubstituted, or cyclo groups: C_1 - C_{30}; C_8- C_{24}; or C_{12} - C_{16}.

[0027] Typically, the groups A may be a linear C_{12} - C_{16} alkyl group.

[0028] In particular, A may be $CH_3(CH_2)_{15}$.

[0029] The ratio of quaternary ammonium nitrogens to nitrogens of amino groups may be selected from any of the following: 0.01% - 100%; 10% - 90%; 30% - 70%; 40% - 60%; 50% - 90% or 60% - 80%. The preferred range is 40% - 90%. A high proportion of quaternary ammonium groups promotes solubilisation of both the polyethylenimine polymer and a hydrophobic drug.

[0030] The parent polyethylenimine compound used to make the polyethylenimine polymer may have an average molecular weight of about 2 - 50kD, or more particularly, of about 10 - 25 kD.

[0031] The polyethylene polymer may have an average molecular weight of about 10 - 25 kD.

[0032] The polyethylenimine polymer may produce hydrophobic domains. Hydrophobic domains are areas of the molecule's self-assembly where hydrophobic compounds or compounds which are poorly soluble in water are able to reside and thus become solubilised with an aqueous disperse phase. The level of hydrophobic modification may be from 0.01 - 50%, 0.1 - 20% or 1 - 10% of amino groups. The preferred level of hydrophobic modification is 1 - 10% of amino groups.

[0033] All possible monomeric subunits in accordance with the structure as defined in formula I are shown in Figure 1: wherein

m is between 0 - 90 %;
n is between 0 - 100 %;
p is between 0 - 50 %;
q is between 0 - 50 %;
u is between 0 - 50 %;
v is between 0 - 50 %;
w is between 0 - 20 %;
x is between 0 - 20 %;
y is between 0 - 20 %; and
z is between 0 - 20 %;
wherein, m + n + p + q + u + v + w + x + y + z = 100%; and
A, R_1, R_2, R_3 and Z are as defined above.

[0034] It should be appreciated that the monomer units m, n, p, q, u, v, w, x, y and z may be arranged in any order.

[0035] The ratios for m, n, p, q, u, v, w, x, y and z are numerical ratios.

[0036] Typically, if m = 0% then n is not equal to 0%.

[0037] Typically, if n = 0% then m is not equal to 0 %.

[0038] Typically, if p = 0% then q + u + v + w + x + y + z does not equal 0%.

[0039] Typically, if q = 0% then p + u + v + w + x + y + z does not equal 0%.

[0040] Typically, if u = 0% then p + q + v + w + x + y + z does not equal 0%.

[0041] Typically, if v = 0% then p + q + u + w + x + y + z does not equal 0%.

[0042] Typically, if w = 0% then x + y + z + n does not equal 0%.

[0043] Typically, if x = 0% then w + y + z + n does not equal 0%.

[0044] Typically, if y = 0% then w + x + z + n does not equal 0%.

[0045] Typically, if z = 0% then w + x + y + n does not equal zero.

[0046] Conveniently, m + n lies between 50 to 100%.

[0047] Conveniently, p + q + u + v lies between 20 to 50%.

[0048] Conveniently, w + x + y + z lies between 0.01 to 10%.

[0049] It is possible that polyethylenime may be linear (n=100) or branched as shown in Figure 1. If n = 0%, however, then m must be equal to a value greater than 0% as this allows for the branched material with no backbone quaternisation on erstwhile secondary amines.

[0050] It is possible that p, q, u, v, w, x, y or z may be equal to 0%. However, the sum total of p, q, u, v, w, x, y and z may be equal to a value greater than 0%, as this allows for the branched compound to be included.

[0051] Alternatively, w, x, y or z may be equal to 0%. However, the sum total of w, x, y or z may not be equal to 0%. This allows for a hydrohobically substituted branched compound.

[0052] Typically, m + n = 60%, w + x + y + z = 6%, and p + q + u + v = 34%. Using these ranges defines the quaternary ammonium cetyl polyethylenimine found in the Example Section of the present application.

[0053] According to a second aspect of the present invention there is provided a method of forming a polyethylenimine polymer according to the first aspect by reacting a polyethylenimine compound formed from the polymerisation of ethylenimine with a first organo halide to form an organo side chain on the polyethylenimine compound, and then a second organo halide to react with an amino group on the polyethyleneimine compound.

[0054] The polyethylenimine used may be branched or linear.

[0055] Branched polyethylenimine may be prepared by the acid catalysed polymerisation of, for example, aziridine (ethyleneimine) (Dick, C.R., Ham, G.E., J. Macromol. Sci. 1970, A4, 1301-1314; von Harpe, A., Petersen, H., Li, Y., Kissel, T., J. Control. Rel. 2000, 69, 309-332). Linear polymers may be prepared by controlling the conditions of polyethylenimine polymerisation (Zhuk, D.S., Gembitsky, P.A., Alexandrovich, A.I., US Patent No. 4,032,480).

[0056] The first organo halide may be any linear or branched, substituted or unsubsituted, or cyclo form of any alkyl, alkenyl, alkynyl, aryl or acyl halide or any hydrophilic halide. The halide may be any of fluoride, chloride, bromide or iodide.

[0057] The organo group of the first organo halide may be selected from any of the following linear or branched, substituted or unsubstituted, or cyclo groups: C_1 - C_{30}; C_8 - C_{24}; or C_{12} - C_{16}.

[0058] Typically, the first organo halide is a linear C_{12} - C_{16} alkyl halide.

[0059] In particular, the first organo halide may be cetyl bromide (e.g. $CH_3(CH_2)_{15}$ Br).

[0060] The second organo halide may be any alkyl, alkenyl, alkynyl, aryl or acyl halide or any hydrophilic halide. The halide may be any of fluoride, chloride, bromide or iodide.

[0061] The organo group of the second organo halide may be selected' from any of the following linear or branched, substituted or unsubstituted, or cyclo groups: C_1 - C_{20}; C_1 - C_6: or C_1.

[0062] Typically, the second organo halide is a linear C_1 - C_6 alkyl halide. In particular, the second organo halide may be methyl iodide.

[0063] The polyethylenimine compound and first organo halide may be mixed in an organic solvent such as tetrahydrofuran, which may then be refluxed. The refluxing may occur in an alcoholic solution of, for example, sodium hydroxide. Cetyl polyethylenimine may then be isolated and may then be reacted with the second organo halide.

[0064] The second organo halide may be added in the presence of, for example, a metal hydroxide (e.g. sodium hydroxide), a metal halide (e.g. sodium iodide) and an alcohol (e.g. methanol).

[0065] The polyethylenimine polymer may then be obtained by washing, dialysis and using an ion exchange column.

[0066] Further quaternisation may be obtained by adding more of the second organo halide.

[0067] The formed polyethylenimine polymer may be that as represented in Figure 1.

[0068] It is also possible to prepare a substituted linear polyethylenimine with the end nitrogens protected, subsequently deprotect the terminal amines and then attach this substituted linear polyethylenimine to the branched molecule and follow the whole conjugation step with a quaternary ammonium step.

[0069] Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.1 M and preferably 0.05 M phosphate buffer or 0.9% w/v saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

[0070] Typically, the ratio of polyethylenimine polymer to pharmaceutically acceptable carrier ranges from any of the following: 0.0001 - 100 w.v., 0. 005 - 50 w.v.; 0.001 - 30 w.v.; 0.001 - 10 w.v.; or 0.01 - 1 w.v.

[0071] The drug may be poorly soluble in aqueous solvents such as water. The drug may be administered to a patient as a solution or a particulate formulation.

[0072] The drug may be selected from any of the following: cyclosporin; steroids such as prednisolone, oestradiol, testosterone; drugs with multicyclic ring structures which lack polar groups such as paclitaxel; and drugs such as etoposide.

[0073] Typically, the ratio of the polyethylenimine polymer to the drug may be selected from any of the following: 0.001 - 100%; 0.1 - 100%; 1 - 100%; 10 - 90%; 30 - 70%.

[0074] The pharmaceutical composition may also comprise a pharmaceutically acceptable carrier.

[0075] Typically, the ratio of polyethylenimine polymer to drug to pharmaceutically acceptable carrier may be in the range of 5 - 20mg : 0.5 - 5mg : 0.5 - 5mL or 5 - 20mg : 0.5 - 5mg : 0.5 - 5g. In particular, the ratio of polyethylenimine polymer to drug to pharmaceutically acceptable carrier may be about 10mg:2mg:1mL or about 10mg:2mg:2g.

[0076] The pharmaceutical composition may be in the form of any of the following: tablets, suppositories, liquid capsule, powder form, or a form suitable for pulmonary delivery.

[0077] When tablets are used for oral administration, typically used carriers include sucrose, lactose, mannitol, maltitol, dextran, corn starch, typical lubricants such as magnesium stearate, preservatives such as paraben, sorbin, antioxidants such as ascorbic acid, α-tocopheral, cysteine, disintegrators or binders. When administered orally as capsules, effective diluents include lactose and dry corn starch. A liquid for oral use includes syrup, suspension, solution and emulsion, which may contain a typical inert diluent used in this field, such as water. In addition, sweeteners or flavours may be contained.

[0078] Suppositories may be prepared by admixing the compounds of the present invention with a suitable non-irritative excipient such as those that are solid at normal temperature but become liquid at the temperature in the intestine and

melt in rectum to release the active ingredient, such as cocoa butter and polyethylene glycols.

[0079] The dose of the polymer can be determined on age, body weight, administration time, administration method, combination of drugs, the level of condition of which a patient is undergoing therapy, and other factors. While the daily does may vary depending on the conditions and body weight of patients, the species of active ingredient, and administration route, in the case of oral use, the daily does may be about 0.1 - 100 mg/person/day, preferably 0.5 - 30 mg/person/day.

Brief Description of the Drawings

[0080] Embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings in which:

Figure 1 is a representation of a polyethyleneimine polymer formed according to the present invention; and
Figure 2 is a Transmission Electron Microscopy (TEM) image of quaternary ammonium cetyl polyethyleneimine (QCPEI2) and cyclosporin nanoparticles.

Examples

Example 1 - Synthesis of Quaternary Ammonium Cetyl Polyethylenimine (QCPEI)

[0081] Alkylation of polyethylenimine was carried out according to a previously reported method (Noding, G., Heitz, W., 1998, Amphiphilic Polyethylenimines Based on Long-Chain Alkyl Bromide Macromolecular Chemistry and Physics 199: 637 - 1644). Briefly, polyethylenimine (M_w = 25kD, 5g) was alkylated by refluxing with cetyl bromide (1.8g) and tetrahydrofuran (50ml) for 48 hours, followed by the addition of an alcoholic solution of sodium hydroxide (4.8g in 25ml methanol), and a further reflux period of 24 hours. Sodium bromide was removed by filtration and the product isolated by evaporation of the solvent, exhaustive dialysis and freeze-drying. 0.6g of cetyl polyethylenimine was then quaternised by reaction with methyl iodide (2.6ml) in the presence of sodium hydroxide (0.23g), sodium iodide (0.28g) and methanol (100ml) for 3 hours at 36°C. The product was isolated by precipitation in ether (400ml), washing with ethanol, exhaustive dialysis of an ethanolic solution and elution through an ion exchange column to isolate the hydrochloride salt.

[0082] A yellow cotton wool like solid which is the quaternary ammonium cetyl polyethyleneimine (QCPEI1) was obtained on freeze drying.

[0083] A further quaternisation of quaternary ammonium cetyl polyethyleneimine (QCPEI1) produced a doubly quaternerised compound, i.e. di-quaternary ammonium cetyl polyethyleneimine (QCPEI2).

Characterisation of Quaternary Ammonium Cetyl Polyethylenimine

[0084] ^1H NMR and ^1H correlation spectroscopy as well as ^{13}C NMR experiments (Bruker, AMX 400 MHz spectrometer, Bruker Instruments UK) were carried out on the quaternary cetyl polyethyleneimine in deuterated methanol. Elemental analysis was carried out on the products using a Perkin Elmer 2400 analyser.

Polymer Aggregation

[0085] The aggregation of an aqueous solution of the polymers was studied using a pyrene probe for hydrophobic domains (see Kalyanasundaram, K., Thomas, J.K., 1977, Environmental Effects on the Vibronic Band Intensities in Pyrene Monomer Fluorescence and the Application to Studies of Micellar Systems, Journal of the American Chemical Society 99: 2039 - 2044). Fluorescence scans (excitation = 340nm) were performed on various concentrations of the polymer dissolved in an aqueous pyrene solution (2μM). The ratio of the intensity of the third and first peaks (I_3/I_1) was used to assess the hydrophobicity of the pyrene environment which is an indirect probe for polymer association.

[0086] Polymer aggregation was also assessed by recording the hypsochromic shift in the UV absorption spectrum of methyl orange (Lieske, A., Jaeger, W., 1999, Block Copolymers Containing Polysoap Blocks, Tenside Surfactants Detergents 36: 155 - 161) in 25μM in 0.02M borate buffer when encapsulated within a hydrophobic environment. UV absorption scans (300 - 600nm) were performed on various concentrations of the polymer dissolved in the methyl orange-borate solution and the wavelength of maximum absorbance noted.

TABLE 1: Quaternary ammonium cetyl polyethyleneimine (QCPEI1) aggregation in aqueous solution as measured by the increase in (I_3/I_1) ratio in the pyrene fluorescence and by the hypsochromic shift in the methyl orange spectra

QCPEI1 I3/I1 ratio (QCPEI1 concentration in mg mL^{-1})	QCPEI1 Methyl Orange wavelength of maximum absorbance (QCPEI1 concentration in mg mL^{-1})	QCPEI2 I3/I1 ratio (QCPEI2 concentration in mg mL^{-1})	QCPEI2 Methyl Orange wavelength of maximum absorbance (QCPEI2 concentration in mg mL^{-1})
0.64 (0)	465(0)	0.61 (0)	465 (0)
0.88 (0.87)	450 (0.50)	0.823 (0.81)	456 (0.55)
0.89 (1.73)	452 (1.52)	0.862 (1.621)	450 (1.63)
0.92 (3.73)	452 (3.73)	0.871 (3.24)	458 (3.70)
0.98 (7.04)	454 (7.80)	0.853 (4.37)	455 (7.85)
		0.926 (6.49)	456 (14.25)

[0087] The synthesis of the cetyl polyethylenimine was confirmed by a proton NMR and assignments were made as follows:

$\delta = 0.87 = CH_3$ (cetyl), $\delta 1.25 = CH_2$ (cetyl), $\delta 1.45 = CH_2$ - N (cetyl), 52.7 - 2.8 = CH_2 - N (cetyl and polyethylenimine). Quaternisation of cetyl polyethylenimine to produce quaternary ammonium cetyl polyethylenimine was confirmed by ^{13}C NMR: $\delta 14.6 = CH_3$(cetyl), $\delta 23.9 = CH_2$(cetyl), $\delta 52.5$ and $54.8 = CH_3(CH_3N^+)$, 558.8 and 63.5 = CH_2N and CH_2N+(polyethylenimine) and ^1H NMR - $\delta 0.90 = CH_3$ (cetyl), $\delta 1.3 = CH_2$ (cetyl), 51.47 = CH_2 (cetyl), $\delta 1.85 = CH_2$ - N (cetyl), 52.5 - 4.7 = CH_2N, CH_2N^+ and CH_3N^+.

[0088] The yields of cetyl polyethylenimine, quaternary polyethyleneimine (QCPEI1) and di-quaternary cetyl polyethyleneimine (QCPEI1) were 67%, 85% and 46%, respectively.

[0089] The degree of cetylation was found to be 5.2% of all amine groups using elemental analysis data. The degree of conversion of amines to quaternary ammonium moieties was approximately 64% for quaternary cetyl polyethylenimine and 81% for di-quaternary cetyl polyethylenimine.

[0090] Both quaternary ammonium polymers aggregate to produce hydrophobic domains in aqueous solution (See Table 1). This is shown by the increase in the I3/I1 values and also by the shift to a lower wavelength of the methyl orange peak. These hydrophobic domains serve to solubilise poorly aqueous soluble (hydrophobic) drugs such as cyclosporin; in the case of the less quaternised variant - QCPEI1 which forms a clear micellar liquid with cyclosporin, when freshly prepared (Table 1), effectively encapsulating cyclosporin within the hydrophobic domains.

Example 2 - Preparation of Quaternary Cetyl Polyethylenimine - Cyclosporin Formulations

[0091] Quaternary cetyl polyethylenimine polymers were dissolved by probe sonication on ice (Soniprep Instruments, UK) followed by the addition of cyclosporin, which was incorporated into the polymer solution by probe sonication. Formulations were stored for up to 13 days and observed for particle formation. Particulate formations were sized by photon correlation spectroscopy, imaged by both transmission electron microscopy (TEM) with negative staining (see Wang, W., Tetley, L., Uchegbu, I.F., 2001. The Level of Hydrophobic Substitution and the Molecular Weight of Amphiphilic Poly-L-Lysine-based Polymers Strongly Affects Their Assembly into Polymeric Bilayer Vesicles, Journal of Colloid and Interface Science 237: 200-207) and freeze fracture electron microscopy (see Uchegbu, I.F., Schatzlein, A.G., Tetley, L., Gray, A.I., Sludden, J., Siddique, S., Mosha, E., 1998, Polymeric Chitosan - Based Vesicles for Drug Delivery, Journal of Pharmacy and Pharmacology 50: 453-458). Clear micellar formulations were filtered with a 0.45μm filter and the filtered formulations assayed by HPLC using a reverse phase Waters Spherisorb ODS column (25cm x 4.6mm), eluted with a water, acetonitrile tert-butyl methyl ether, orthophosphoric acid (350:600:50:1). Detection was by UV(λ=210nm).

Table 2: QCPEI-cyclosporin formulations

Formulation	Initial Appearance	Initial Mean Particle Size (nm)	% Recovery of cyclosporin from micellar solutions(a)		Mean Particle Size (nm)	
			Freshly prepared (mean ± s.d.)	After storage (2-8°C) for 90 days (mean ± s.d.)	Storage (2-8°C) for 4 days followed by exposure to room temperature for 15 min	Storage (2-8°C) for 3 days followed by exposure to 37°C for 15 min
QCPEI1	Clear liquid	-	78.7±8.14 (n=3)	93.3±6.60 (n=4)	558 (n=3)	608 (n=6)
QCPEI2	Colloidal	310 (n=4)	-	-	377(n=1)	512(n=3)
(a) Initial Concentration = 2mg mL^{-1} n Denotes number of formulations assayed.						

[0092] [In Table 2 the blank boxes (represented with a "-") represent particulate formulations, which cannot be assayed in the same way as micellar formulations].

[0093] As shown in Table 1 both quaternary ammonium polymers (i.e. QCPEI1 and QCPEI2) aggregate to produce hydrophobic domains in aqueous solutions. These hydrophobic domains serve to solubilise cyclosporin. In the case of the less quaternerised variant - QCPEI1 forms a clear micellar liquid with cyclosporin, when freshly prepared, effectively encapsulating cyclosporin within hydrophobic domains. However, as shown in Table 2, the polymer exhibits a lower critical solution temperature and becomes less hydrated with increase in temperature resulting in aggregation of the polymeric micelles to form nanoparticles. Furthermore, Table 2 shows storage of QCPEI1 at refrigeration temperature preserved the micellar formulation. The micellar formulation is preserved as analysis of the optically clear samples after storage for 90 days shows that there is no precipation of cyclosporin.

[0094] In contrast to QCPEI1, the doubly quaternarised compound QCPEI2, which is less water soluble than QCPEI1 initially formed stable nanoparticles with cyclosporin. Figure 2 shows that the double quaternarised compound (QCPEI2) does not form micelles with cyclosporin. The size bar shows that the aggregates formed are too large to be micelles although the image could show an aggregate of lots of micelles. These will still be technically nanoparticles as the formulation is not optically clear.

[0095] Although the polymer forms micelles within which cyclosporin is solubilised, the polymer exhibits a lower critical solution temperature and becomes less hydrated with increase in temperature resulting in aggregation of the polymeric micelles to form nanoparticles after exposure to elevated temperatures (i.e. removal from the fridge, Table 2). However, storage of QCPEI1 at refrigeration temperature preserved the micellar formulation (Table 2) and there was no conversion of the micelles into nanoparticles. In contrast to QCPEI1, the doubly quaternised compound QCPEI2, which is less water soluble than QCPEI1, initially formed stable nanoparticles with cyclosporin (Figure 2, Table 2) and does not form the micelles with cyclosporin.

Example 3 - Oral Administration of Quaternary Cetyl Polyethylenimine-Cyclosporin Formulations

[0096] Groups of male Wistar rats (n=4 i.e. the group size, weight = 200 - 220g) were fasted for 12 hours before dosing and subsequently dosed intragastrically (10mg kg^{-1}) with an optically clear quaternary cetyl polyethylenimine (QCPEI1) - cyclosporin formulation (10:2); a particulate quaternary cetyl polyethylenimine (QCPEI2) - Cyclosporin (10:2) formulation; Neoral (Registered Trademark) or water. Neoral is a microemulsion formulation of cyclosporin manufactured and marketed by Novartis.

[0097] Blood was taken from the tail vein of these anaesthetised rats at 1 hour, 4 hours and 24 hours after dosing. Plasma was separated by centrifugation at 1000g and stored at -20°C until analysis could be performed on the samples. Cyclosporin was measured in the plasma samples using a monoclonal antibody radioimmunoassay kit (Cyclo-Trac SP-Whole Body Radioimmunoassay Kit) supplied by Diasorin, UK.

Table 3: Blood Levels Following Oral Cyclosporin Dosing

Time	Formulations ngL^{-1} of cyclosporin in blood		
	Neoral®	QCPEI1	QCPEI2
1h	1525±267*	583±284	748±482
4h	1521±163	1179±360	1387±539
24h	346±37	315±95	295±45
*** = statistically significant difference between groups at the same time point ($p<0.05$)**			

[0098] The oral QCPEI1 formulations were well tolerated in rats with no gross adverse events recorded. Plasma levels at the 4 hour time point from the oil free QCPEI formulations were indistinguishable from peak levels obtained using Neoral (Registered Trademark), although Neoral (Registered Trademark) was absorbed faster than the QCPEI formulations shown in Table 3. The amphiphilic polyethyleneimine polymer therefore promotes the absorption of a poorly soluble drug such as cyclosporin.

[0099] Within the 37°C environment of the gut lumen it is assumed, although not wishing to be bound by theory, that the narrow particle formulation prevails for both polymers and that these nanoparticles experience the gradual loss of cationic micellar aggregates still encapsulating their hydrophobic payload. As cationic polymers are known to facilitate transport across epithelial membranes and across cell membranes, these micellar aggregates may also facilitate the intestinal absorption of cyclosporin. The disassociation of the nanoparticle into single micellar aggregates results in the delayed absorption when compared to the oil containing formulation.

Example 4 - Oral Delivery of Cyclosporin 2

[0100] This Example examines the effect of intermediate and low molecular weight quaternary ammonium hexadecyl polyethylenimine on the oral delivery of cyclosporine A.

Materials

[0101] Polyethylenimine (Mw = 10kD) was supplied by Polysciences, UK. Polyethylenimine (Mw = 1.8kD), hexadecyl bromide, methyl iodide and sodium iodide were all obtained from Sigma-Aldrich, Co., UK. Ethanol, diethyl ether and tetrahydrofuran were supplied by the Department of Pure and Applied Chemistry, University of Strathclyde.

Methods

[0102] Intermediate molecular weight quaternary ammonium cetyl PEI with two different levels of quaternary ammonium modification ($Q1_{10}$ and $Q2_{10}$) were synthesised by reacting polyethylenimine (PEI, Mw = 10kD) with both cetyl bromide and methyl iodide as described for QCPEI1 and QCPEI2 respectively in Example 1. Low molecular weight quaternary ammonium cetyl PEI with a high level of quaternary ammonium modification ($Q2_{1.8}$) was synthesised by reaction of PEI (Mw = 1.8kD) with both cetyl bromide and methyl iodide as described for QCPEI2 in Example 1. $Q1_{10}$, $Q2_{10}$, and $Q2_{1.8}$ cyclosporine (2mg mL^{-1}) formulations, each containing 10mg mL^{-1} of the respective amphiphilic PEI were prepared as described in Example 2.

[0103] Male Wistar rats (mean weight = XXg [WPC PLEASE COMPLETE], n = 4) were dosed orally with QCPEI1, $Q1_{10}$, $Q2_{10}$ or Neoral formulations of cyclosporine (7.5mg kg^{-1}). Blood was then sampled at various time intervals and cyclosporine analysed in the sampled blood using the radioimmunoassay procedure described in Example 3. In a separate experiment male Wistar rats (mean weight = XXg [WPC PLEASE COMPLETE], n = 4) were dosed orally with $Q2_{10}$, $Q2_{1.8}$, or Neoral formulations of cyclosporine (10mg kg^{-1}). A further group was dosed with a dispersion of cyclosporine (10mg kg^{-1}) in water which was shaken just prior to administration. Blood was sampled from these 4 groups of animals at various time intervals and cyclosporine analysed in blood using the radioimmunoassay procedure described in Example 3.

Results

[0104]

Table 4: Blood levels of cyclosporine after dosing animals orally with 7.5mg Kg^{-1} cyclosporine

Formulation	Blood levels (ng mL^{-1}, n = 4, mean ± s.d.)		
	1h	4h	24h
$Q1_{10}$	615 ± 351*	854 ± 376	73 ± 38
$Q2_{10}$	1050 ± 456	1163 ± 326	95 ± 19
QCPEI1	576 ± 320*	799 ± 481	84 ± 44
Neoral	1496 ± 447	989 ± 301	150 ± 68
*Statistically significantly different from Neoral ($p < 0.05$)			

Table 5: Blood levels of cyclosporine after dosing animals orally with 10mg Kg^{-1} cyclosporine

Formulation	Blood levels (ng mL^{-1}, n = 4, mean ± s.d.)		
	1h	4h	24h
$Q2_{1.8}$	889 ± 336*	1677 ± 840	461 ± 153#
$Q2_{10}$	1213 ± 196* #	1865 ± 516#	565 ± 115#
Cyclosporine dispersion in water	439 ± 345*	617 ± 277*	88 ± 43
Neoral	2026 ± 209#	1915 ± 158#	475 ± 133#
*Statistically significantly different from Neoral ($p < 0.05$). #statistically significantly different from cyclosporine dispersion in water.			

Comment on Results

[0105] At the 7.5mg kg-1 dose level $Q2_{10}$ had an equivalent bioavailability with Neoral while $Q1_{10}$ and QCPEI1 delivered less cyclosporine via the oral route after 1h when compared to Neoral, although cyclosporine levels equivalent to Neoral were delivered at the 4h and 24h time points by both $Q1_{10}$ and QCPEI1.

[0106] At the 10mg kg-1 dose level, all formulations delivered less cyclosporine than Neoral at the 1h time point although $Q2_{10}$ improved the absorption of cyclosporine when compared to cyclosporine dispersion in water. At the 4h time point both $Q2_{10}$ and $Q2_{1.8}$ were bioequivalent with Neoral whereas due to the high standard deviations obtained with $Q2_{1.8}$, this formulation was statistically indistinguishable from the cyclosporine dispersion in water. At the 24h time point all formulations resulted in a greater absorption of cyclosporine when compared to the cyclosporine dispersion in water.

[0107] It is clear that polyethylenimine amphiphiles are able to promote the absorption of cyclosporine.

Example 5: Stability of Cyclosporin Solutions

[0108] This Example relates to assessing the stability of quaternary ammonium polyethylenimine - cyclosporine formulations.

Materials

[0109] Polyethylenimine (Mw = 10kD) was supplied by Polysciences, UK. Polyethylenimine (MW = 25kD), hexadecyl bromide, methyl iodide and sodium iodide were all obtained from Sigma-Aldrich, Co., UK. Ethanol, diethyl ether and tetrahydrofuran were supplied by the Department of Pure and Applied Chemistry, University of Strathclyde.

Methods

[0110] $Q1_{10}$ was synthesised by reacting polyethylenimine (PEI, Mw = 10kD) with both cetyl bromide and methyl iodide as described for QCPEI1 in Example 1. QCPEI1 was also synthesised as described in Example 1. $Q1_{10}$ and QCPEI1 Formulations of cyclosporine (2mg mL^{-1}) containing 10mg mL^{-1} of the amphiphilic PEIs were prepared as

described in Example 2.

[0111] Formulations were then stored in stoppered glass containers at refrigeration temperature (2 - 8ºC). At various time intervals aliquots were sampled, filtered through a 0.45μm filter and analysed by high performance liquid chromatography (HPLC). Filtered cyclosporine samples (20μL) dissolved in acetonitrile, water (1: 1) were injected onto a Waters Spherisorb 5μm, 4.6mm X 250mm column (Waters Instruments, UK) maintained at 80°C with a Jones Chromatography Column Heater model 7971 by means of a Waters 717 autosampler and a Waters 515 isocratic pump. The mobile phase was acetonitrile: water: tert-butylmethyl-ether: phosphoric acid (600:350:50:1) at a flow rate of 1.2mL min^{-1}. Peak detection was via a Waters 486 variable wavelength UV detector with the wavelength set at 210nm and data was collected using a Waters 746 data module. A standard curve was prepared using solutions of the drug (1 - 10μg mL^{-1}).

Results

[0112]

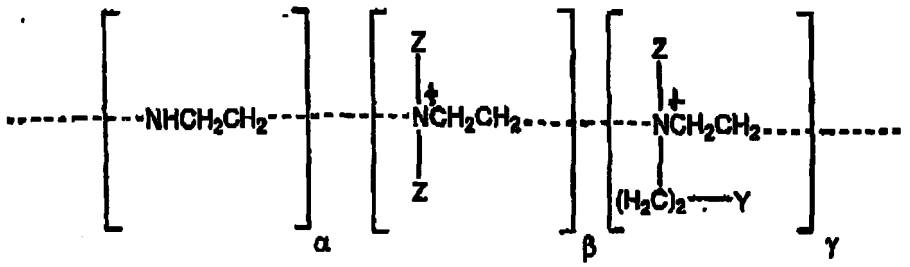
Time Point (days)	QCPEI1	$Q1_{10}$
0	78.7 ± 8.1	80.7 ± 17.7
7	84.6 ± 3.1	74.0 ± 8.1
41	93.7 ± 8.8	81.9 ± 3.6
109	91.0 ± 6.3	79.0 ± 0.6
181	84.4 ± 2.9	82.0 ± 2.3
281	89.4 ± 0.42	79.0 ± 1.4

Comment on Results

[0113] Over a 9 month period the level of cyclosporine recovered from amphiphilic PEI formulations $Q1_{10}$ and QCPEI1 did not differ appreciably from the original levels, indicating that these formulations were stable when stored for 9 months at refrigeration temperatures.

Claims

1. A pharmaceutical composition of a poorly soluble drug and amphiphilic polyethylenimine polymer, wherein the drug has an aqueous solubility of less than 0.1 mg per millilitre of solvent at a temperature of about 15-20°C, wherein the polymer contains hydrophilic substituents that comprise quaternary nitrogen groups and hydrophobic substituents as represented by the formula:



wherein

α is between 0 to 90%;
β is between 0 to 100%;
γ is between 0 to 50%;
and α + β + γ = 100%; and

and further wherein:

each Z group is independently hydrogen or a hydrophobic substituent, wherein the hydrophobic substituent is a linear or branched, substituted or unsubstituted or a cyclic group; and
Y is a hydrophilic substituent.

2. A pharmaceutical composition according to claim 1, wherein:

(a) the monomer units identified with α, β and γ form any arrangement in the polyethylenimine polymer;
(b) the arrangement of the α, β and γ units are random or in a block copolymer form.

3. A pharmaceutical composition according to claim 1 or claim 2, wherein the polyethylenimine polymer is linear or branched.

4. A pharmaceutical composition according to any one of preceding claims, wherein in the polyethylenimine polymer:

(a) at least one of the Z groups is selected from: an alkyl group, an alkenyl group, and alkynyl group, an aryl group or an acyl group; or
(b) at least one of the Z groups is selected a C_1-C_{20} group, a C_1-C_{12} group, a C_1-C_6 group or a C_1 group; or
(c) at least one of the Z groups is a C_1-C_4 linear alkyl group.

5. A pharmaceutical composition according to any one of the preceding claims, wherein in the polyethylenimine polymer, Y represents $-NH_2$, -NHA, $-N^+R_1R_2R_3$ or $-N^+R_1R_2A$, and wherein each of R_1, R_2, or R_3 is independently a linear or branched, substituted or unsubstituted, or cyclo group selected from: alkyl, alkenyl, alkynyl, aryl, acyl, hydroxy alkyl, hydroxy acyl, polyethylene glycol or a sugar; and A is a C_1-C_{30} linear or branched, substituted or unsubstituted or cyclo group.

6. A pharmaceutical composition according to claim 5, wherein in the polyethylenimine polymer, there are between 1 and 3 $R_{1\text{-}3}$ substituents on any single nitrogen.

7. A pharmaceutical composition according to claim 5 or claim 6, wherein:

(a) each of R_1, R_2 and R_3 is independently a C_1-C_{20} group, a C_1-C_{12} group, a C_1-C_6 group or a C_1 group; or
(b) each of R_1, R_2 and R_3 is a C_1-C_4 linear alkyl group; or
(c) each of R_1, R_2 and R_3 is CH_3.

8. A pharmaceutical composition according to claim 5, wherein in the polyethylenimine polymer:

(a) the A group is a linear or branched, substituted or unsubstituted, or cyclo groups selected from a C_1-C_{30}, a C_8-C_{24} group or a C_{12}-C_{16} group; or
(b) the A group is a linear C_{12}-C_{16} alkyl group; or
(c) the A group is $CH_3(CH_2)_{15}$.

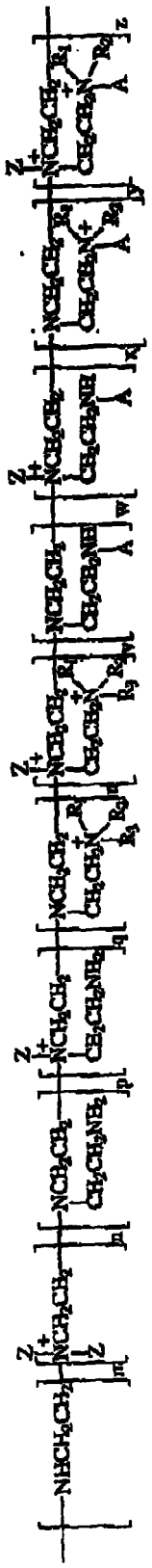
9. A pharmaceutical composition according to any one of the preceding claims, wherein in the polyethylenimine polymer, the ratio of quaternary ammonium nitrogens to nitrogens of amino groups is selected from: 0.01% - 100%; 10% - 90%; 30% - 70%; 40% - 60%; 50% - 90%; 60% - 80% or 40% - 90%.

10. A pharmaceutical composition according to any one of the preceding claims, wherein the polyethylenimine polymer has an average molecular weight of about 10 - 25 kD.

11. A pharmaceutical composition according to any one of the preceding claims, wherein the polyethylenimine polymer produces hydrophobic domains.

12. A pharmaceutical composition according to claim 11, wherein the level of hydrophobic modification is from about 0.01 - 50%, about 0.1 - 20% or about 1 - 10% of amino groups.

13. A pharmaceutical composition according to any preceding claim, wherein the polyethylenimine polymer comprises monomeric subunits in accordance with the structure as defined in formula I shown below:



wherein

m is between 0 - 90 %;
n is between 0 - 100 %;
p is between 0 - 50 %;
q is between 0 - 50 %;
u is between 0 - 50 %;
v is between 0 - 50 %;
w is between 0 - 20 %;
x is between 0 - 20 %;
y is between 0 - 20 %; and
z is between 0 - 20 %;

wherein, m + n + p + q + u + v + w + x + y + z = 100%.

14. A pharmaceutical composition according to claim 13, wherein the monomer units m, n, p, q, u, v, w, x, y and z are arranged in any order.

15. A pharmaceutical composition according to claim 13 or claim 14, any preceding claim wherein:

when m = 0% then n is not equal to 0%;
when n = 0% then m is not equal to 0 %;
when p = 0% then q + u + v + w + x + y + z does not equal 0%;
when q = 0% then p + u + v + w + x + y + z does not equal 0%;
when u = 0% then p + q + v + w + x + y + z does not equal 0%;
when v = 0% then p + q + u + w + x + y + z does not equal 0%;
when w = 0% then x + y + z + n does not equal 0%;
when x = 0% then w + y + z + n does not equal 0%;
when y = 0% then w + x + z + n does not equal 0%;
when z = 0% then w + x + y + n does not equal zero.

16. A pharmaceutical composition according to any one of claims 13 to 15, wherein:

(a) m + n lies between 50 to 100%; and/or
(b) p + q + u + v lies between 20 to 50%; and/or
(c) wherein w + x + y + z lies between 0.01 to 10%; and/or
(d) p, q, u, v, w, x, y or z are equal to 0%; and/or
(e) the sum total of p, q, u, v, w, x, y and z is equal to a value greater than 0% thereby forming a branched compound; and/or
(f) w, x, y or z are equal to 0%; and/or
(g) m + n = 60%, w + x + y + z = 6%, and p + q + u + v = 34%.

17. A pharmaceutical composition comprising a polyethylenimine polymer according to any one of the preceding claims, further comprising a pharmaceutically acceptable carrier.

18. A pharmaceutical composition according to claim 17, wherein the weight to volume ratio (w.v.) of the polyethylenimine polymer to the pharmaceutically acceptable carrier ranges from any of the following: 0.0001 - 100 w.v., 0.005 - 50 w.v.; 0.001 - 30 w.v.; 0.001 - 10 w.v.; or 0.01 - 1 w.v.

19. A pharmaceutical composition according to claim 17 or claim 18 comprising a drug, wherein the drug is selected from any of the following: cyclosporin; steroids such as prednisolone, oestradiol, testosterone; drugs with multicyclic ring structures which lack polar groups such as paclitaxel; and drugs such as etoposide.

20. A pharmaceutical composition according to any one of claims 17 to 19, wherein the ratio of the polyethylenimine polymer to the drug is selected from any of the following: 0.001-100%; 0.1-100%; 1-100%; 10-90% or 30-70%.

21. A pharmaceutical composition according to any one of claims 17 to 20, wherein the ratio of polyethylenimine polymer to drug to pharmaceutically acceptable carrier is selected from:

(a) about 5-20 mg : 0.5-5 mg : 0.5-5 mL; or
(b) 5-20 mg : about 5-5 mg : 0.5-5 g; or
(c) about 10 mg:2 mg: 1 mL; or
(d) about 10 mg:2 mg: 2 g.

22. A pharmaceutical composition according to claim 21, wherein the pharmaceutical composition is in the form of tablets, suppositories, liquid capsule powder form or a form suitable for pulmonary delivery.

23. A pharmaceutical composition according to any one of claims 17 to 22, wherein the drug is formulated for oral delivery

24. Use of a polyethylenimine polymer as defined in any one of claims 1 to 16 for the preparation of a medicament wherein the polymer is formulated with a poorly soluble drug, wherein the drug has an aqueous solubility of less than 0.1 mg per millilitre of solvent at a temperature of about 15-20°C.

25. The use according to claim 24, wherein the poorly soluble drug is selected from any of the following: cyclosporin; steroids; drugs with multicyclic ring structures which lack polar groups; etoposide.

26. A method of forming a polyethylenimine polymer defined according to any one of claims 1 to 16, the method comprising reacting a polyethylenimine compound formed from the polymerisation of ethylenimine with a first organo halide to form an organo side chain on the polyethylenimine compound, and then reacting a second organo halide with an amino group on the polyethyleneimine polymer.

27. A method according to claim 26, wherein the ethylenimine is branched or linear.

28. A method according to claim 26 or claim 27, wherein the first organo halide is any linear or branched, substituted or unsubsituted, or cyclo form of any alkyl, alkenyl, alkynyl, aryl or acyl halide or any hydrophilic halide.

29. A method according to claim 28, wherein the first organo halide is:

(a) a C_1-C_{20} group, a C_1-C_{12} group, a C_1-C_6 group or a C_1 group; or
(b) a linear C_{12}-C_{16} alkyl halide; or
(c) cetyl bromide.

30. A method according to any one of claims 26 to 29, wherein the second organo halide is a linear or branched, substituted or unsubsituted, or cyclo form of an alkyl, alkenyl, alkynyl, aryl or acyl halide or a hydrophilic halide.

31. A method according to claim 30, wherein the second organo halide is:

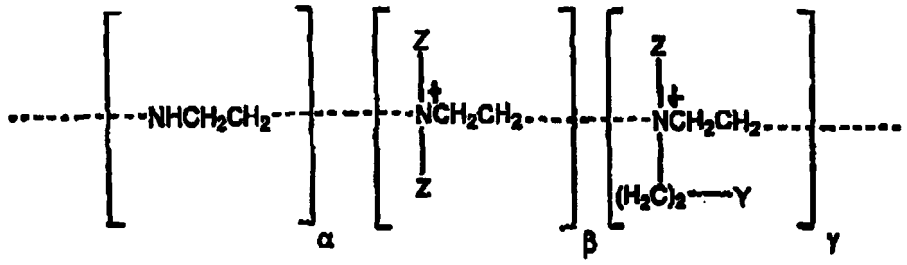
(a) a C_1-C_{20} group, a C_1-C_{12} group, a C_1-C_6 group or a C_1 group; or
(b) a linear C_1-C_6 alkyl halide; or
(c) methyl iodide.

32. A method according to any of claims 28 to 31, wherein:

(a) the polyethylenimine compound and first organo halide are mixed in an organic solvent, which is then refluxed in an alcoholic solution of sodium hydroxide, and cetyl polyethylenimine is then isolated and reacted with the second organo halide; or
(b) the second organo halide is added in the presence of a metal hydroxide, a metal halide and an alcohol.

Patentansprüche

1. Pharmazeutische Zusammensetzung eines schwach löslichen Arzneistoffes und eines amphiphilen Polyethylenimin-Polymeren, wobei der Arzneistoff eine wässrige Löslichkeit von weniger als 0,1 mg pro Milliliter Lösungsmittel bei einer Temperatur von etwa 15-20 °C aufweist, wobei das Polymere hydrophile Substituenten, die quaternäre Stickstoffgruppen umfassen, und hydrophobe Substituenten der folgenden Formel enthält:



wobei

α 0 bis 90 % beträgt;
β 0 bis 100 % beträgt;
γ 0 bis 50 % beträgt;

und α + β + γ = 100%; und
wobei ferner:

die Gruppen Z jeweils unabhängig voneinander Wasserstoff oder einen hydrophoben Substituenten bedeuten, wobei es sich beim hydrophoben Substituenten um eine lineare oder verzweigte, substituierte oder unsubstituierte oder cyclische Gruppe handelt; und
Y einen hydrophilen Substituenten bedeutet.

2. Pharmazeutische Zusammensetzung nach Anspruch 1, wobei

(a) die mit α, β und γ bezeichneten Monomereinheiten eine beliebige Anordnung im Polyethylenimin-Polymeren bilden;
(b) die Anordnung der α-, β- und γ-Einheiten statistisch oder in Blockcopolymerform ist.

3. Pharmazeutische Zusammensetzung nach Anspruch 1 oder 2, wobei das Polyethylenimin-Polymere linear oder verzweigt ist.

4. Pharmazeutische Zusammensetzung nach einem der vorstehenden Ansprüche, wobei im Polyethylen-Polymeren:

(a) mindestens eine der Z-Gruppen ausgewählt ist aus: einer Alkylgruppe, einer Alkenylgruppe und Alkinylgruppe, einer Arylgruppe oder einer Acylgruppe; oder
(b) mindestens eine der Z-Gruppen ausgewählt ist aus einer C_1-C_{20}-Gruppe, einer C_1-$_{12}$-Gruppe, einer C_1-C_6-Gruppe oder einer C_1-Gruppe; oder
(c) mindestens eine der Z-Gruppen eine lineare C1-C4-Alkylgruppe bedeutet.

5. Pharmazeutische Zusammensetzung nach einem der vorstehenden Ansprüche, wobei im Polyethylenimin-Polymeren Y die Bedeutung -NH2, -NHA, $-N^+R_1R_2R_3$ oder $-N^+R_1R_2A$ hat und wobei jeder der Reste R_1, R_2 oder R_3 unabhängig voneinander eine lineare oder verzweigte, substituierte oder unsubstituierte oder Cyclogruppe bedeutet, ausgewählt aus: Alkyl, Alkenyl, Alkinyl, Aryl, Acyl, Hydroxyalkyl, Hydroxyacyl, Polyethylenglycol oder ein Zucker; und A eine lineare oder verzweigte, substituierte oder unsubstituierte C_1-C_{30}-Gruppe oder Cyclogruppe bedeutet.

6. Pharmazeutische Zusammensetzung nach Anspruch 5, wobei im Polyethylenimin-Polymeren sich 1 bis 3 $R_{1\text{-}3}$-Substituenten an einem beliebigen einzelnen Stickstoffatom befinden.

7. Pharmazeutische Zusammensetzung nach Anspruch 5 oder 6, wobei:

(a) jeder der Reste R_1, R_2 und R_3 unabhängig voneinander eine C_1-C_{20}-Gruppe, eine C_1-C_{12}-Gruppe, eine C_1-C_6-Gruppe oder eine C_1-Gruppe bedeutet; oder
(b) jeder der Reste R_1, R_2 und R_3 eine lineare C_1-C_4-Alkylgruppe bedeutet; oder
(c) jeder der Reste R_1, R_2 und R_3 die Bedeutung CH_3 hat.

8. Zusammensetzung nach Anspruch 5, wobei im Polyethylenimin-Polymeren:

(a) die A-Gruppe eine lineare oder verzweigte, substituierte oder unsubstituierte Gruppe oder Cyclogruppen bedeutet, die aus C_1-C_{30}-, C_8-C_{24}- oder C_{12}-C_{16}-Gruppen ausgewählt sind; oder
(b) die A-Gruppe eine lineare C_{12}-C_{16}-Alkylgruppe bedeutet; oder
(c) die A-Gruppe $CH_3(CH_2)_{15}$ bedeutet.

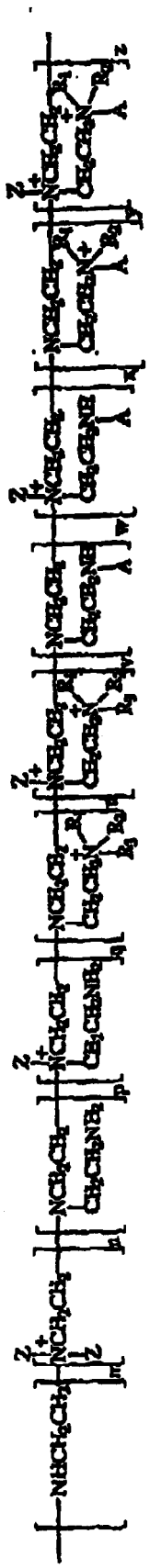
9. Pharmazeutische Zusammensetzung nach einem der vorstehenden Ansprüche, wobei im Polyethylenimin-Polymeren das Verhältnis der quaternären Ammonium-Stickstoffatome zu Stickstoffatomen von Aminogruppen ausgewählt ist aus: 0,01%-100%; 10%-90%; 30%-70%; 40%-60%; 50%-90%; 60%-80% oder 40%-90%.

10. Pharmazeutische Zusammensetzung nach einem der vorstehenden Ansprüche, wobei das Polyethylenimin-Polymere ein durchschnittliches Molekulargewicht von etwa 10 bis 25 kD aufweist.

11. Pharmazeutische Zusammensetzung nach einem der vorstehenden Ansprüche, wobei das Polyethylenimin-Polymere hydrophobe Domänen erzeugt.

12. Pharmazeutische Zusammensetzung nach Anspruch 11, wobei der Grad der hydrophoben Modifikation etwa 0,01-50%, etwa 0,1-20% oder etwa 1-10% der Aminogruppen betrifft.

13. Pharmazeutische Zusammensetzung nach einem der vorstehenden Ansprüche, wobei das Polyethylenimin-Polymere monomere Untereinheiten gemäß der Struktur in der nachstehend angegebenen Formel I umfasst:



wobei

m 0 - 90 % ausmacht;
n 0 - 100 % ausmacht;
p 0 - 50 % ausmacht;
q 0 - 50 % ausmacht;
u 0 - 50 % ausmacht;
v 0 - 50 % ausmacht;
w 0 - 20 % ausmacht;
x 0 - 20 % ausmacht;
y 0 - 20 % ausmacht; und
z 0 - 20 % ausmacht;

wobei m + n + p + q + u + v + w + x + y + z = 100%

14. Pharmazeutische Zusammensetzung nach Anspruch 13, wobei die Monomereinheiten m, n, p, q, u, v, w, x, y und z in einer beliebigen Reihenfolge angeordnet sind.

15. Pharmazeutische Zusammensetzung nach Anspruch 13 oder 14, wobei:

wenn m = 0 % gilt, n nicht gleich 0 % ist;
wenn n = 0 % gilt, m nicht gleich 0 % ist;
wenn p = 0 % gilt, q + u + v + w + x + y + z nicht gleich 0 % sind;
wenn q = 0 % gilt, p + u + v + w + x + y + z nicht gleich 0 % sind;
wenn u = 0 % gilt, p + q + v + w + x + y + z nicht gleich 0 % sind;
wenn v = 0 % gilt, p + q + u + w + x + y + z nicht gleich 0 % sind;
wenn w = 0 % gilt, x + y + z + n nicht gleich 0 % sind;
wenn x = 0 % gilt, w + y + z + n nicht gleich 0 % sind;
wenn y = 0 % gilt, w + x + z + n nicht gleich 0 % sind;
wenn z = 0 % gilt, w + x + y + n nicht gleich 0 % sind.

16. Pharmazeutische Zusammensetzung nach einem der Ansprüche 13 bis 15, wobei

(a) m + n 50 bis 100 % betragen; und/oder
(b) p + q + u + v 20 bis 50 % betragen; und/oder
(c) wobei w + x + y + z 0,01 bis10 % betragen; und/oder
(d) p, q, u, v, w, x, y oder z 0 % betragen; und/oder
(e) die Summe aus p, q, u, v, w, x, y und z einen Wert ergeben, der größer als 0 % ist, wodurch eine verzweigte Verbindung gebildet wird; und/oder
(f) w, x, y oder z 0 % ergeben; und/oder
(g) m + n = 60%, w + x + y + z = 6% und p + q + u + v = 34%.

17. Pharmazeutische Zusammensetzung, umfassend ein Polyethylenimin-Polymeres nach einem der vorstehenden Ansprüche, ferner umfassend einen pharmazeutisch verträglichen Träger.

18. Pharmazeutische Zusammensetzung nach Anspruch 17, wobei das Verhältnis von Gewicht/Volumen (w.v.) des Polyethylenimin-Polymeren zum pharmazeutisch verträglichen Träger in einem der folgenden Bereiche liegt: 0,0001-100 w.v., 0,005-50 w.v., 0,001-30 w.v., 0,001-10 w.v. oder 0,01-1 w.v.

19. Pharmazeutische Zusammensetzung nach Anspruch 17 oder 18, umfassend einen Arzneistoff, wobei der Arzneistoff in beliebiger Weise aus der folgenden Gruppe ausgewählt ist: Cyclosporin; Steroide, wie Prednisolon, Östradiol, Testosteron; Arzneistoffe mit multicyclischen Ringstrukturen, bei denen polare Gruppen fehlen, wie Paclitaxel; und Arzneistoffe, wie Etoposid.

20. Pharmazeutische Zusammensetzung nach einem der Ansprüche 17 bis 19, wobei das Verhältnis des Polyethylenimin-Polymeren zum Arzneistoff in beliebiger Weise aus einem der folgenden Bereiche ausgewählt ist: 0,001-100%; 0,1-100%, 1-100%; 10-90% oder 30-70%.

21. Pharmazeutische Zusammensetzung nach einem der Ansprüche 17 bis 20, wobei das Verhältnis vom Polyethylenimin-Polymeren zum Arzneistoff und dem pharmazeutisch verträglichen Träger ausgewählt ist aus:

(a) etwa 5-20 mg : 0,05-5 mg : 0,5-5 ml; oder
(b) 5-20 mg : etwa 5-5 mg : 0,5-5 g; oder
(c) etwa 10 mg : 2 mg : 1 ml; oder
(d) etwa 10 mg : 2 mg : 2 g.

22. Pharmazeutische Zusammensetzung nach Anspruch 21, wobei die pharmazeutische Zusammensetzung in Form von Tabletten, Suppositorien, flüssigkeitsgefüllten Kapseln, in Pulverform oder in einer Form, die für die pulmonale Abgabe geeignet ist, vorliegt.

23. Pharmazeutische Zusammensetzung nach einem der Ansprüche 17 bis 22, wobei der Arzneistoff für die orale Abgabe zubereitet ist.

24. Verwendung eines Polyethylenimin-Polymeren gemäß einem der Ansprüche 1 bis 16 zur Herstellung eines Arzneimittels, wobei das Polymere mit einem schwach löslichen Arzneistoff zubereitet wird, wobei der Arzneistoff eine wässrige Löslichkeit von weniger als 0,1 mg pro Milliliter Lösungsmittel bei einer Temperatur von etwa 15-20 °C aufweist.

25. Verwendung nach Anspruch 24, wobei der schwach lösliche Arzneistoff aus einem beliebigen der folgenden Arzneistoffe ausgewählt wird: Cyclosporin; Steroide; Arzneistoffe mit multicyclischen Ringstrukturen, bei denen polare Gruppen fehlen; Etoposid.

26. Verfahren zur Bildung eines Polyethylenimin-Polymeren gemäß einem der Ansprüche 1 bis 16, wobei das Verfahren folgendes umfasst: Umsetzung einer Polyethylenimin-Verbindung, die durch Polymerisation von Ethylenimin mit einem ersten Organohalogenid unter Bildung einer Organoseitenkette an der Polyethylenimin-Verbindung gebildet worden ist, und anschließende Umsetzung eines zweiten Organohalogenids mit einer Aminogruppe am Polyethylenimin-Polymeren.

27. Verfahren nach Anspruch 26, wobei das Ethylenimin verzweigt oder linear ist.

28. Verfahren nach Anspruch 26 oder 27, wobei es sich beim ersten Organohalogenid um ein beliebiges lineares oder verzweigtes, substituiertes oder unsubstituiertes oder cyclisches Alkyl-, Alkenyl-, Alkinyl-, Aryl- oder Acylhalogenid oder um ein beliebiges hydrophiles Halogenid handelt.

29. Verfahren nach Anspruch 28, wobei es sich beim ersten Organohalogenid um:

(a) eine C_1-C_{20}-Gruppe, eine C_1-C_{12}-Gruppe, eine C_1-C_6-Gruppe oder eine C_1-Gruppe; oder
(b) ein lineares C_{12}-C_{16}-Alkylhalogenid; oder
(c) Cetylbromid handelt.

30. Verfahren nach einem der Ansprüche 26 bis 29, wobei es sich beim zweiten Organohalogenid um eine lineares oder verzweigtes, substituiertes oder unsubstituiertes oder cyclisches Alkyl-, Alkenyl-, Alkinyl-, Aryl- oder Acylhalogenid oder um ein hydrophiles Halogenid handelt.

31. Verfahren nach Anspruch 30, wobei es sich beim zweiten Organohalogenid um

(a) eine C_1-C_{20}-Gruppe, eine C_1-C_{12}-Gruppe, eine C_1-C_6-Gruppe oder eine C_1-Gruppe; oder
(b) ein lineares C_1-C_6-Alkylhalogenid; oder
(c) Methyljodid handelt.

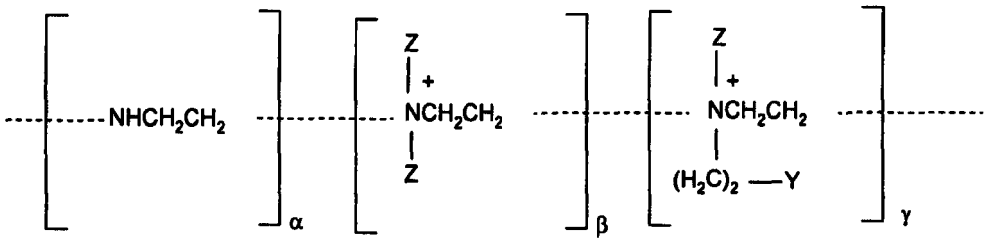
32. Verfahren nach einem der Ansprüche 28 bis 31, wobei:

(a) die Polyethylenimin-Verbindung und das erste Organohalogenid in einem organischen Lösungsmittel vermischt werden, das anschließend in einer alkoholischen Lösung von Natriumhydroxid unter Rückfluss erwärmt wird, wobei anschließend das Cetylpolyethylenimin isoliert und mit dem zweiten Organohalogenid umgesetzt wird; oder

(b) das zweite Organohalogenid in Gegenwart eines Metallhydroxids, eines Metallhalogenids und eines Alkohols zugesetzt wird.

Revendications

1. Composition pharmaceutique d'un médicament médiocrement soluble et d'un polymère de polyéthylèneimine amphiphile, dans laquelle le médicament présente une solubilité aqueuse inférieure à 0,1 mg par millilitre de solvant à une température d'environ 15-20°C, dans laquelle le polymère contient des substituants hydrophiles qui comprennent des groupes d'azote quaternaire et des substituants hydrophobes comme représenté par la formule :



dans laquelle

α est compris entre 0 et 90 % ;
β est compris entre 0 et 100 % ;
γ est compris entre 0 et 50 % ;
et α + β + γ = 100 % ; et
et de plus dans laquelle :
chaque groupe Z est indépendamment un atome d'hydrogène ou un substituant hydrophobe, dans laquelle le substituant hydrophobe est un groupe linéaire ou ramifié, substitué ou non substitué ou un groupe cyclique ; et
Y est un substituant hydrophile.

2. Composition pharmaceutique selon la revendication 1, dans laquelle :

(a) les unités monomères identifiées avec α, β et γ forment un arrangement quelconque dans le polymère de polyéthylèneimine ;
(b) l'arrangement des α, β et γ unités est aléatoire ou dans une forme de copolymère séquencé.

3. Composition pharmaceutique selon la revendication 1 ou la revendication 2, dans laquelle le polymère de polyéthylèneimine est linéaire ou ramifié.

4. Composition pharmaceutique selon l'une quelconque des revendications précédentes, dans laquelle dans le polymère de polyéthylèneimine :

(a) au moins un des groupes Z est choisi parmi :

un groupe alkyle, un groupe alcényle, un groupe alcynyle, un groupe aryle ou un groupe acyle ; ou

(b) au moins un des groupes Z est choisi parmi un groupe en C_1-C_{20}, un groupe en C_1-C_{12}, un groupe en C_1-C_6 ou un groupe en C_1 ; ou
(c) au moins un des groupes Z est un groupe alkyle linéaire en C_1-C_4.

5. Composition pharmaceutique selon l'une quelconque des revendications précédentes, dans laquelle dans le polymère de polyéthylèneimine, Y représente $-NH_2$, -NHA, $-N^+R_1R_2R_3$ ou $-N^+R_1R_2A$, et dans laquelle chacun des R_1, R_2 ou R_3 est indépendamment un groupe linéaire ou ramifié, substitué ou non substitué, ou un groupe cyclo choisi parmi : un groupe alkyle, alcényle, alcynyle, aryle, acyle, hydroxyalkyle, hydroxyacyle, polyéthylèneglycol ou un sucre ; et A est un groupe linéaire ou ramifié, substitué ou non substitué ou un groupe cyclo en C_1-C_{30},

6. Composition pharmaceutique selon la revendication 5, dans laquelle dans le polymère de polyéthylèneimine, il y a entre 1 et 3 substitutants R_{1-3} sur tout azote unique.

7. Composition pharmaceutique selon la revendication 5 ou la revendication 6, dans laquelle :

(a) chacun parmi R_1, R_2 et R_3 est indépendamment un groupe en C_1-C_{20}, un groupe en C_1-C_{12}, un groupe en C_1-C_6 ou un groupe en C_1 ; ou
(b) chacun parmi R_1, R_2 et R_3 est un groupe alkyle linéaire en C_1-C_4 ; ou
(c) chacun parmi R_1, R_2 et R_3 est CH_3.

8. Composition pharmaceutique selon la revendication 5, dans laquelle dans le polymère de polyéthylèneimine :

(a) le groupe A est un groupe linéaire ou ramifié, substitué ou non substitué ou un groupe cyclo choisi parmi un groupe en C_1-C_{30}, un groupe en C_8-C_{24} ou un groupe en C_{12}-C_{16} ; ou
(b) le groupe A est un groupe alkyle en C_{12}-C_{16} linéaire ; ou
(c) le groupe A est $CH_3(CH_2)_{15}$.

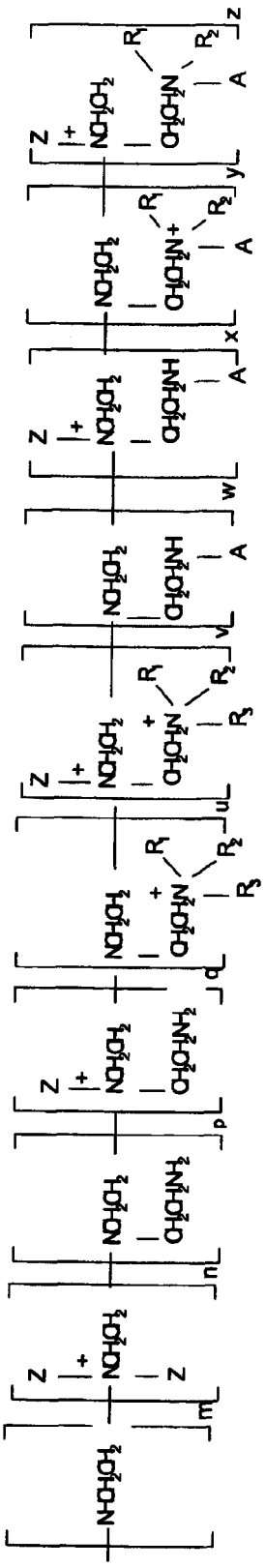
9. Composition pharmaceutique selon l'une quelconque des revendications précédentes, dans laquelle dans le polymère de polyéthylèneimine, le rapport des azotes d'ammonium quaternaire aux azotes des groupes amino est choisi parmi : 0,01 % - 100 %, 10 % - 90 % ; 30 % - 70 % ; 40 % - 60 % ; 50 - 90 % ; 60 % - 80 % ou 40 % - 90 %.

10. Composition pharmaceutique selon l'une quelconque des revendications précédentes, dans laquelle le polymère de polyéthylèneimine présente une masse moléculaire moyenne d'environ 10-25 kD.

11. Composition pharmaceutique selon l'une quelconque des revendications précédentes, dans laquelle le polymère de polyéthylèneimine produit des domaines hydrophobes.

12. Composition pharmaceutique selon la revendication 11, dans laquelle le niveau de modification hydrophobe est d'environ 0,01 - 50 %, d'environ 0,1-20 % ou d'environ 1 - 10 % de groupes amino.

13. Composition pharmaceutique selon l'une quelconque des revendications précédentes, dans laquelle le polymère de polyéthylèneimine comprend des sous-unités monomères selon la structure comme défini dans la formule (I) représentée ci-dessus :



dans laquelle

m est compris entre 0 et 90 % ;
n est compris entre 0 et 100 % ;
p est compris entre 0 et 50 % ;
q est compris entre 0 et 50 % ;
u est compris entre 0 et 50 % ;
v est compris entre 0 et 50 % ;
w est compris entre 0 et 20 % ;
x est compris entre 0 et 20 % ;
y est compris entre 0 et 20 % ; et
z est compris entre 0 et 20 % ;
dans laquelle m + n + p + q + u + v + w + x + y + z = 100 %.

14. Composition pharmaceutique selon la revendication 13, dans laquelle les unités monomères m, n, p, q, u, v, w, x, y et z sont disposées dans un ordre quelconque.

15. Composition pharmaceutique selon la revendication 13 ou la revendication 14, une revendication précédente quelconque, dans laquelle :

lorsque m est égal à 0 % alors n n'est pas égal à 0 % ;
lorsque n est égal à 0 % alors m n'est pas égal à 0 % ;
lorsque p est égal à 0 % alors q + u + v + w + x + y + z n'est pas égal à 0 % ;
lorsque q est égal à 0 % alors p + u + v + w + x + y + z n'est pas égal à 0 % ;
lorsque u est égal à 0 % alors p + q + v + w + x + y + z n'est pas égal à 0 % ;
lorsque v est égal à 0 % alors p + q + u + w + x + y + z n'est pas égal à 0 % ;
lorsque w est égal à 0 % alors x + y + z + n n'est pas égal à 0%;
lorsque x est égal à 0 % alors w + y + z + n n'est pas égal à 0%;
lorsque y est égal à 0 % alors w + x + z + n n'est pas égal à 0%;
lorsque z est égal à 0 % alors w + x + y + n n'est pas égal à 0%;

16. Composition pharmaceutique selon l'une quelconque des revendications 13 à 15, dans laquelle :

(a) m + n est compris entre 50 et 100 % ; et/ou
(b) p + q + u + v est compris entre 20 et 50 % ; et/ou
(c) dans laquelle w + x + y + z est compris entre 0,01 et 10 % ; et/ou
(d) p, q, u, v, w, x, y ou z sont égaux à 0 % ; et/ou
(e) la somme totale de p, q, u, v, w, x, y et z est égale à une valeur supérieure à 0 % formant par là un composé ramifié ; et/ou
(f) w, x, y ou z sont égaux à 0 % ; et/ou
(g) m + n = 60 %, w + x + y + z = 6 %, et p + q + u + v = 34 %.

17. Composition pharmaceutique comprenant un polymère de polyéthylèneimine selon l'une quelconque des revendications précédentes, comprenant de plus un support pharmaceutiquement acceptable.

18. Composition pharmaceutique selon la revendication 17, dans laquelle le rapport masse à volume (m.v.) du polymère de polyéthylèneimine au support pharmaceutiquement acceptable est compris dans l'un des intervalles suivants : 0,0001 - 100 m.v., 0,005 - 50 m.v. ; 0,001 - 30 m.v. ; 0,001 - 10 m.v. ; ou 0,01 - 1 m.v.

19. Composition pharmaceutique selon la revendication 17 ou la revendication 18 comprenant un médicament, dans laquelle le médicament est choisi parmi l'un quelconque des suivants : la cyclosporine ; des stéroïdes, tels que la prednisolone, l'oestradiol, la testostérone ; des médicaments avec des structures de noyaux multicycliques qui manquent de groupes polaires, tels que le paclitaxel ; et des médicaments tels que l'étoposide.

20. Composition pharmaceutique selon l'une quelconque des revendications 17 à 19, dans laquelle le rapport du polymère de polyéthylèneimine au médicament est choisi parmi l'un quelconque des suivants : 0,001 - 100 % ; 0,1 - 100 % ; 1 - 100 % ; 10-90 % ou 30-70%.

21. Composition pharmaceutique selon l'une quelconque des revendications 17 à 20, dans laquelle le rapport du polymère de polyéthylèneimine au médicament au support pharmaceutiquement acceptable est choisi parmi :

(a) environ 5 - 20 mg : 0,05 - 5 mg : 0,5 - 5 ml ; ou
(b) 5 - 20 mg : environ 5 - 5 mg : 0,5 - 5 g ; ou
(c) environ 10 mg : 2 mg : 1 ml ; ou
(d) environ 10 mg : 2 mg : 2 g.

22. Composition pharmaceutique selon la revendication 21, dans laquelle la composition pharmaceutique est dans la forme de comprimés, de suppositoires, dans une forme de poudre en capsule liquide ou dans une forme appropriée pour une administration pulmonaire.

23. Composition pharmaceutique selon l'une quelconque des revendications 17 à 22, dans laquelle le médicament est formulé pour une administration orale.

24. Utilisation d'un polymère de polyéthylèneimine selon l'une quelconque des revendications 1 à 16 pour la préparation d'un médicament dans lequel le polymère est formulé avec un médicament médiocrement soluble, dans laquelle le médicament présente une solubilité aqueuse inférieure à 0,1 mg par millilitre de solvant à une température d'environ 15-20°C.

25. Utilisation selon la revendication 24, dans laquelle le médicament médiocrement soluble est choisi parmi l'un quelconque des suivants : la cyclosporine ; des stéroïdes ; des médicaments avec des structures de noyaux multicycliques qui manquent de groupes polaires ; l'étoposide.

26. Procédé de formation d'un polymère de polyéthylèneimine défini selon l'une quelconque des revendications 1 à 16, le procédé comprenant la réaction d'un composé de polyéthylèneimine formé à partir de la polymérisation d'éthylèneimine avec un premier organohalogénure pour former une chaîne latérale organo sur le composé de polyéthylèneimine et la réaction subséquente d'un second organohalogénure avec un groupe amino sur le polymère de polyéthylèneimine.

27. Procédé selon la revendication 26, dans lequel l'éthylèneimine est ramifiée ou linéaire.

28. Procédé selon la revendication 26 ou la revendication 27, dans lequel le premier organohalogénure est une forme linéaire ou ramifiée, substituée ou non substituée ou cyclo d'un halogénure quelconque d'un halogénure d'alkyle, d'alcényle, d'alcynyle, d'aryle ou d'acyle quelconque ou d'un halogénure hydrophile quelconque.

29. Procédé selon la revendication 28, dans lequel le premier organohalogénure est :

(a) un groupe en C_1-C_{20}, un groupe en C_1-C_{12}, un groupe en C_1-C_6 ou un groupe en C_1 ; ou
(b) un halogénure d'alkyle en C_{12}-C_{16} linéaire ; ou
(c) le bromure de cétyle.

30. Procédé selon l'une quelconque des revendications 26 à 29, dans lequel le second organohalogénure est une forme linéaire ou ramifiée, substituée ou non substituée, ou cyclo d'un halogénure d'alkyle, d'alcényle, d'alcynyle, d'aryle ou d'acyle ou d'un halogénure hydrophile.

31. Procédé selon la revendication 30, dans lequel le second organohalogénure est :

(a) un groupe en C_1-C_{20}, un groupe en C_1-C_{12}, un groupe en C_1-C_6 ou un groupe en C_1 ; ou
(b) un halogénure d'alkyle en C_1-C_6 linéaire ; ou
(c) l'iodure de méthyle.

32. Procédé selon l'une quelconque des revendications 28 à 31, dans lequel :

(a) le composé de polyéthylèneimine et le premier organohalogénure sont mélangés dans un solvant organique, lequel est ensuite mis sous reflux dans une solution alcoolique d'hydroxyde de sodium, et la cétylpolyéthylèneimine est ensuite isolée et réagit avec le second organohalogénure ; ou
(b) le second organohalogénure est ajouté en présence d'un hydroxyde de métal, d'un halogénure de métal et

d'un alcool.

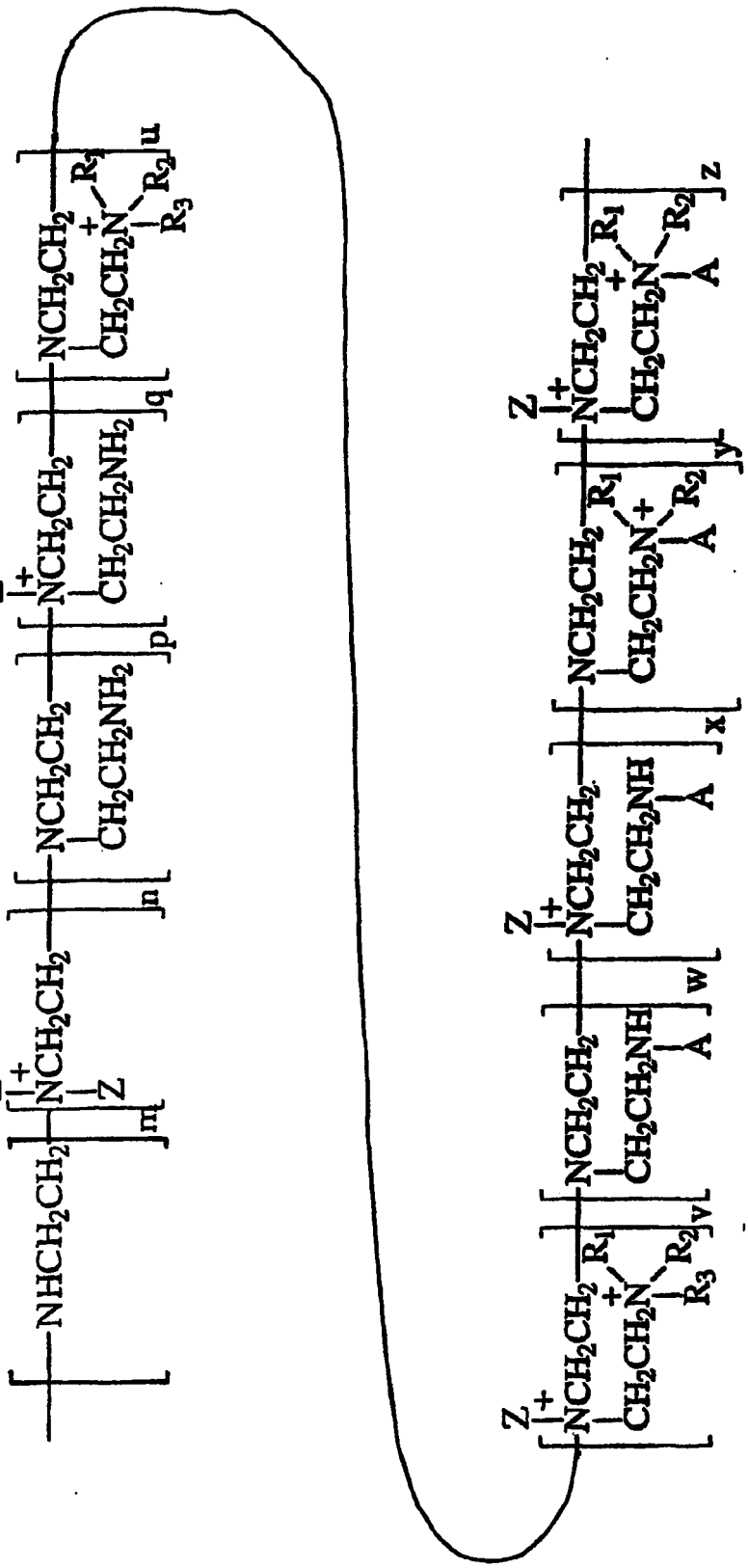


Figure 1

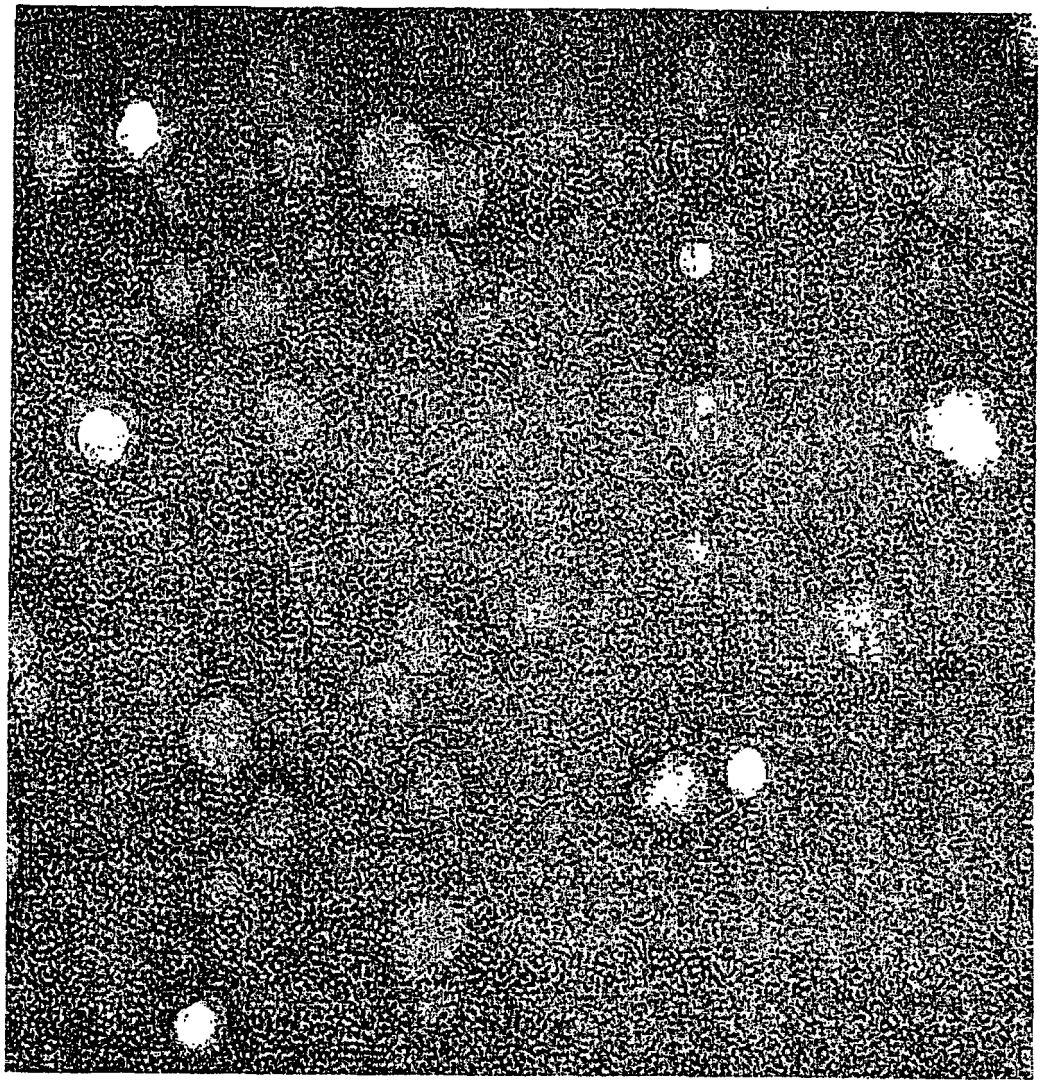


FIGURE 2

REFERENCES CITED IN THE DESCRIPTION

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